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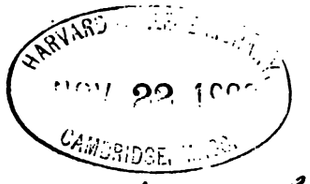

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THE DISTRIBUTION OF BACTERIUM COLI COMMUNE

By HARRIETTE CHICK, B.Sc., 1851 Exhibition Scholar

From the School of Pathology, Thompson Yates Laboratories, Liverpool

THIS work was undertaken at the suggestion of Professor Boyce, in order to study Bacterium coli commune, with special reference to its distribution. The significance and origin of the bacillus, when present, especially in sources of water supply, were points also which required clearing up, viz. :—How far its presence might be considered as an index of pollution by sewage, in which fluid the bacillus is known to exist in large numbers. The experiments described later in this paper were undertaken with these two objects :—(1) To examine carefully, material well-known to be polluted, and, at the same time, material that might be considered unpolluted, in order to see if the respective presence or absence of the B. coli could be established. It was especially important to definitely ascertain whether, for instance, the brooks and rivers of the water supply of a large city like Liverpool normally contained the B. coli commune, and, if so, in what numbers. (2) To see if there was evidence of multiplication of this bacillus in water, soil, dust, etc., if any of these became contaminated; or whether, on the other hand, the presence of the bacillus might be taken as indicative of recent pollution.

A method had to be adopted by means of which the presence of B. coli commune could be demonstrated and a quantitative estimation made. A solid medium was found to be much more convenient than a liquid one; and a carbolized nutrient agar, of which a full description is given later, incubated at a high temperature, has been found most useful for isolating and enumerating the bacillus.

It has frequently been stated that, since B. coli commune is widely distributed in nature and is often to be found in air, soil, and water from all sources, its presence cannot be taken as direct evidence of pollution. Lehmann* describes an experiment in which he found, in yeast, a bacillus closely resembling the typical B. coli commune, and brings this forward as a reason why the presence of such a bacillus is not a sure sign of contamination. Other authors, Kruse,† Stoddart,‡ Lunt,§ think that these examples of B. coli commune, found in unpolluted material, are probably only 'coli-like' bacilli, and that the presence of the typical, intestinal, B. coli is rarer than has been supposed, although various organisms, resembling it more or less closely, are

* *Centralbl. f. Bakt.* xv, p. 350.
† *Zeitschrift für Hygiene*, xvii, p. 1.
‡ *Journ. of Path.* 1897, p. 422.
§ *Proc. Jenn. Inst.*, 2nd series, 1899, p. 249.

undoubtedly to be found very widespread in nature. These 'coli-like' organisms have been described at length by various observers, most recently by LUNT, in the paper already referred to, and by GORDON.* THEOBALD SMITH† is of the opinion that many statements of the widespread distribution of B. coli are without experimental foundation. He considers that, when found, its presence may be regarded as due to direct contamination. But actual experiments on this subject are rarely met with, and it therefore seemed worth while to make systematic experiments with polluted and unpolluted material, to see what significance might be attributed to the presence of B. coli commune. I have taken care to identify the bacillus whenever met with, and I have not found that 'coli-like' bacilli are at all general; among a very great number of examples of B. coli isolated, there were only three that were not typical. In the Tables the presence of B. coli commune always refers to the typical bacillus; and, I think, these investigations also tend to show that its presence is a good index of pollution.

Samples of rain, snow, hail, air, dust, grains and many foods were found to be free from B. coli commune—virgin moorland soil, similarly. Streams and rivulets, not obviously polluted, showed an absence of the bacillus in most cases in the quantities analyzed; while, in the cases in which it was present, it was there in very small quantity. Water from pipes draining the land was also generally free from it. Sewage and sewage effluents, on the other hand, contain B. coli commune in great numbers, and its presence is also easily demonstrated in rivers polluted by sewage.

These experiments have also shown the efficiency of sand and earth as filtering material for removing the bacillus, and have added a few more facts to what is already known‡ as to the vitality of B. coli commune under unfavourable circumstances. These confirm the conclusions of other observers, that, owing to its low vitality, the B. coli undergoes little multiplication in nature outside its normal habitat, viz., in water, dust, etc. This being so, the presence of the bacillus may be taken as evidence not only of pollution but also of recent pollution.

I wish to take this opportunity of expressing my indebtedness to Professor BOYCE for his invaluable help and kind advice during the course of this investigation.

I. METHOD

The first method tried, which proved to be unsatisfactory, was the well-known one which consists in incubating sewage, etc., properly diluted, with a peptone-salt broth, to which enough phenol has been added to make its concentration in the mixture equal to 1 in 1,000. In the case both of sewage and of sewage effluents the growth obtained consisted largely of liquefying organisms, which outgrew the B. coli commune, although it must undoubtedly have been present. Often, after passage

* *Journ. of Path.*, 1897, p. 438.

† *13th Ann. Report of State Board of Health for New York*, 1893, p. 712.

‡ Frankland and Marshall Ward, *Proc. Roy. Soc.*, 1893, p. 315. Wallieżek, *Centralbl. f. Bakt.* xv, p. 949. Von Freudeureich *Centralbl. f. Bakt.* xx, p. 522.

through a second culture in broth carbolized in the same proportion, a pure culture was obtained by stroking on agar; in some cases, however, the culture so obtained was obviously mixed. Table I gives the details of the former cases—10 samples of crude sewage from West Derby, Chorley, and Dewsbury, yielded pure cultures, all of which were liquefying organisms which also coagulated milk, and 4 of which were spore-bearing. Out of 29 cases of sewage effluents examined, 9 only gave a pure culture of B. coli commune, while 9 gave a liquefying organism, and in 11 cases no growth at all was obtained.

TABLE I

Description of sample	No. of samples examined	No. surviving 1st incubation, 24 hrs. at 42° C. 1 c.c. in 50 c.c. containing 39 c.c. sterile water and 10 c.c. broth carbolized in the proportion of 5 in 1,000	No. surviving 2nd incubation, 24 hrs. at 42° C. 1 drop of latter added to 10 c.c. broth carbolized in the proportion of 1 in 1,000	No. giving liquefying organism, which also coagulated milk, but gave no indol or gas in glucose gelatine	No. giving a spore-bearing liquefying organism	No. giving an organism which agreed with B. coli commune, showing coagulation of milk, indol, gas in glucose gelatine, and no liquefaction, motile bacillus
West Derby Sewage...	5	5	5	5	2	
Dewsbury Sewage ...	3	3	3	3	2	
Chorley Sewage ...	2	2	2	2		
Total Sewages...	10	10	10	10	4	
Leeds Dibdin (No. 4) Bed Effluent ...	1	1	1			1
Leeds Lime Bed Effluent ...	2	2	2	2		
Leeds Cameron Bed Effluent ...	2	2	2	1		1
West Derby Farm Land Effluent ...	17	7	6	4		2
Dewsbury Effluent ...	2	2	2			2
Chorley Tank Effluent	1	1	1	1		
Chorley Filter Effluent	2	2	2			2
Ashton Hall (L'pool) (Experimental) 1st Effluent ...	1	1	1			1
(Experimental) 2nd Effluent ...	1	1	1	1		
Total Sewage Effluents ...	29	19	18	9		9
Total ...	39	29	28	19	4	9

In the method which I finally adopted, nutrient agar, carbolized in the proportion of 1 in 1,000, was used as a medium for the isolation of the B. coli. I first undertook a series of experiments to determine how great a proportion of phenol might be used without inhibiting too much the growth of the B. coli itself. Control plates of ordinary uncarbolized agar were employed for comparison, and the inoculations were made from pure cultures of B. coli commune. I found that the presence of 2 parts of phenol in 1,000 parts of the medium rendered it quite sterile and 1.75 parts rendered it nearly so; 1.5 parts of phenol exercised great inhibition (Plate I, Fig. 4), and 1.33 parts caused the colonies to be distinctly smaller and fewer in number (Fig. 3). It appeared that 1 part of phenol in 1,000 parts of the medium was the limiting quantity which could safely be employed (Fig. 2).

The medium is prepared as follows:—Nutrient agar is prepared in the usual way, and filtered. While still melted one gram of pure phenol is added to each 900 c.c. of the liquid, which is at once distributed, 9 c.c. being introduced by means of a Lode's distributer into each test tube. These should have been previously plugged and sterilized. The tubes containing the carbolized agar are sterilized for twenty minutes, in a KOCH's sterilizer, at 100°, and this has always been found to render tubes and medium quite sterile.

That there is no loss of carbolic acid during this sterilization has been proved in the following way:—Tubes of agar, containing phenol in the proportion of 5 in 1,000, were placed in a KOCH's sterilizer; the end of the tube was closed by an india-rubber stopper and delivery tube, the other end of which dipped beneath the surface of water in another test tube. After 20 minutes the water in the receiving test tube, and washings from the delivery tube, were tested with ferric chloride and found to give no trace of colouration, this test having previously been proved delicate enough to demonstrate the presence of phenol in water in a solution containing only .5 parts per 1,000. A 4 per cent. solution of phenol in water, similarly treated, gave the same result; and, even when boiled, gave no trace of phenol in the distillate. Hence one may assume that no phenol is lost during the sterilization, nor during subsequent melting of the tubes before plating.

When a sample is to be analyzed tubes of carbolized agar, prepared as above stated, are melted by being plunged into boiling water, and are then placed in a large water bath, the temperature of which remains constant at 45° to 47° C. One cubic centimetre of the sample, or of the diluted sample, is added to the melted tube, which is then poured. By this addition the concentration of the phenol in the tube is lowered from 1 in 900 to 1 in 1,000. The plates are incubated at 42° C. for 48 hours, and then are examined, experience having shown that practically no more colonies develop after that time.

It was at first feared that the numbers of B. coli on this medium might be diminished, and experiments were made to see if this were so. Pure cultures of

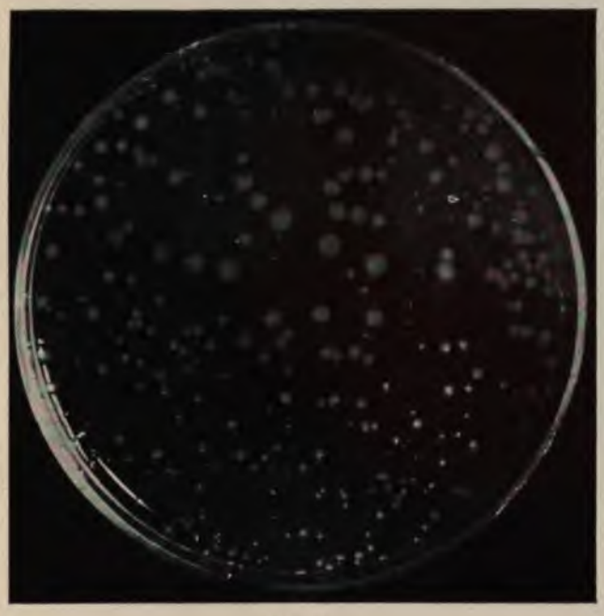


FIG. 1

FIG. 2

FIG. 3

FIG. 4

Photographs of plates made from a pure cultivation of *B. coli commune*, after 48 hours' incubation at 42° C., approximately the same number of organisms being introduced in each case.

Fig. 1—Ordinary nutrient agar.
Fig. 2 " " " carbolized in proportion of 1 in 1000.
Fig. 3 " " " " " " 1.3 in 1000.
Fig. 4 " " " " " " 1.5 in 1000.

B. coli, in broth or peptone-salt solution, were diluted with sterile tap-water (1 in 1,000,000 or 1 in 10,000,000) and plates made of 1 c.c. Comparison was made with ordinary agar, and it was found that, when freshly made dilutions of fresh cultures were used, there was no diminution in numbers on the carbolized medium (Experiments 1, 5, 6, Table II). In Experiment 4 an old culture was used, and in Experiments 2 and 3 old dilutions were used. In this latter case the numbers of B. coli, counted on ordinary agar, decreased in the dilution from 3,505 per c.c., on the day the dilution was made, to 500 two days later, and to 81 at the end of three days. At the same time there was great diminution in the numbers on carbolized agar as compared with those on ordinary agar. It would seem as though—the vitality of the bacillus being lowered by remaining so long in water—it was unable to withstand the carbolized medium. This method, then, gives the number of robust individuals rather than the total number; this, I think, is a point in its favour. It may also be seen from

TABLE II

Experiment No.	Date	Description of culture and dilution	Temperature	† No. of colonies on ordinary agar plates	† No. of colonies on carbolized agar plates
1	March 24, 1900	48 hrs. culture of B. coli commune, diluted 1 in 1,000,000, sterile water and 1 c.c. taken	*37° C.	3505½	5307
			*42° C.	3187	2790
2	March 26, 1900	Dilution of Experiment 1, diluted yet 1 in 100, and 1 c.c. taken	37° C.	5	2
			42° C.	2	0
3	March 27, 1900	Dilution of Experiment 1 used	37° C.	81	30
			42° C.	61	3
4	March 28, 1900	8 days peptone-salt culture, diluted 1 in 10,000,000, and 1 c.c. taken	37°	11	5
			42°	16	4
5	April 6, 1900	24 hrs. broth culture, diluted 1 in 10,000,000, and 1 c.c. taken	37°	469	585
			42°	438	422
			20°		
			(1) 3 days incub	455	0
			(2) 4 days incub.	505	403
6	April 7, 1900	24 hrs. broth culture, diluted 1 in 100,000,000, and 1 c.c. taken	37°	89	104
			42°	114	119
			20°		
			(1) 3 days	147	0
			(2) 23 days	157	84

* All plates incubated at 37° or 42° were counted after 48 hrs. incubation.

† Numbers refer to the mean of three enumerations in each case.

Table II that, when incubated at a low temperature, the carbolic acid in the medium has a very marked inhibitive effect (see Experiments 5 and 6), even when no such effect can be observed at a higher temperature.

If B. coli commune is present in fair quantity, and a high dilution is used, as in sewage, the plates yield an almost pure culture of the bacillus and the numbers are easily counted. The plates also give the very characteristic smell which a culture of B. coli upon agar always has. The appearance of the colonies, both large and small, on the surface and in the depth of the medium is always very characteristic, and can usually be at once identified, especially under the low power of the microscope; the typical colony from these plates has, with two exceptions, always proved to be a typical B. coli commune. In material, such as river water, soil, &c., B. coli commune is present, if at all, in very small quantity, and a very low dilution, or none at all, is used. Here the plates by no means show pure cultures of B. coli; they have to be very carefully examined and separate colonies cultivated, in order to be sure whether they are B. coli or not. The commonest organisms occurring along with B. coli, or in its place, are certain micrococci, one of which agrees with M. candicans, and some spore-bearing organisms of the 'subtilis' type. These, if small and in the depth, may look a little like colonies of B. coli, but can be at once detected as different when grown on agar. Others, however, even in such cases, have an appearance at once distinguishable by hairy mycelium-like outgrowths. Constant use has been made of subcultures on the various media to test the B. coli, when found, to see if it is typical. With these precautions the medium has been found useful to demonstrate the presence of B. coli and its quantity with fair accuracy, even in these latter cases.

The B. coli isolated was in almost every case typical. For example, in experiments from January 12th to February 24th, 1900, made for the most part on river waters, &c., which were not highly polluted, 87 bacilli, resembling B. coli, were isolated and their characteristics observed; 84 were typical B. coli commune; one did not coagulate milk, and two gave no gas in glucose gelatine, although they showed typical coagulation of milk. These three bacilli were, in many other respects, similar to the typical colon bacillus. As to the importance of the various tests in deciding whether a bacillus is B. coli commune or not, a few words are necessary. The coagulation of milk has been considered a necessary property of typical B. coli, and many authors have considered that a form, which only differed in not possessing it, was a separate variety. Pioskowski,* however, considers it to be by no means a specific property, but one which can be modified by previous environment. For example, B. coli obtained from his urine media failed to coagulate milk; but if grown many times on ordinary agar, and then in milk, would give the usual coagulation. I have found that all the examples of B. coli isolated gave coagulation of milk, with the exception of the

* *Centralbl. f. Bakt.* xix, p. 686.

one instance already noted. A good deal, however, depends on the character of milk used; it is very necessary that it should not have reached too high a temperature during sterilization. Hence it is best to sterilize three times in KOCH's sterilizer at 100° C., rather than in the autoclave. In the latter case, I found frequently that no coagulation took place, in cases in which it was very marked in milk, which had not been heated above 100°. All the examples of B. coli isolated gave indol to a greater or less degree; whether this test, also, is as useful as has been supposed has been questioned.*

The occurrence of fermentation, formation of gas and acid, in media containing certain sugars seems to be the first and most important of these characteristics; this is what one would expect, as the reaction appears to be the simplest. For example, in a glucose-gelatine shake culture, when the gas is once produced, its

TABLE II*a*

Description	Date	Dilution	No. on Plate	No. per c.c.
West Derby Sand Bed Effluent	Dec. 11, 1899	·01	1 { 258 2 { 272 3 { 276	25,800 27,200 27,600
West Derby Sand Bed Effluent (another sample)	Dec. 11, 1899	·01	1 { 158 2 { 225 3 { 193	15,800 22,500 19,200
Effluent from West Derby Dibdin Bed, filtered through experimental Filters.				
No. 1 Filter ...	Dec. 12, 1899	undiluted	1 { 18 2 { 18 3 { 31	
No. 2 Filter ...	Dec. 12, 1899	undiluted	1 { 11 2 { 13	
No. 3 Filter ...	Dec. 12, 1899	undiluted	1 { 8 2 { 10 3 { 5	
No. 4 Filter ...	Dec. 12, 1899	undiluted	1 { 14 2 { 15 3 { 16	
Manchester Crude Sewage ...	Dec. 12, 1899	·01	1 { 44 2 { 53 3 { 37	†4,400 5,300 3,370
Open Septic Tank Liquor, Manchester	Dec. 12, 1899	·01	1 { 26 2 { 27 3 { 34	2,600 2,700 3,400
West Derby Dibdin Bed Effluent	Nov. 8, 1899	·01	1 { 114 2 { 105 3 { 111	11,400 10,500 11,100
West Derby Dibdin Bed Effluent	Nov. 9, 1899	·01	1 { 34 2 { 37 3 { 35 4 { 33	3,400 3,700 3,500 3,300

* A. W. Peckham, *Abstr. Centralbl. f. Bakt*, xxiii, p. 986.

† A very low figure for crude sewage.

evidences will remain free from any visible alteration for any length of time. The formation of indol and the coagulation of milk seem to result from much more complicated reactions, and hence are much more likely to be influenced by external conditions. A more exclusive property of B. coli commune than the fermentation of glucose is the fermentation of lactose; this separates it, for example, from B. enteritidis (GÄRTNER), which bacillus it closely resembles.* I should add that of the 84 examples of typical B. coli referred to on page 6, 75 were inoculated into lactose-broth, and all that were thus tested showed fermentation of lactose as well as of glucose. In the former case the test was made by inoculating with the organisms fermentation tubes containing broth, from which all other sugars had been excluded, and to which 1 per cent. lactose had been added. Of the three 'coli-like' organisms, mentioned on page 6, the first produced neither acid nor gas in lactose broth in 48 hours, and thus resembled B. enteritidis (GÄRTNER), while the two others produced acid but no gas in the same medium, even after 12 days.

Except for the three cases already noted, the B. coli isolated have always proved to be typical. The other organisms appearing upon the carbolized agar plates could easily be distinguished from B. coli commune. Out of 183 such organisms which were isolated, 163 were sharply marked off by such unmistakable characteristics, as spore-bearing or a coccus form, while the remaining 20 were unlike, though in a less noticeable manner. Thus, with a few exceptions, such as those noted above, I have not come across examples of the 'coli-like' organisms often described and said to have been found widespread in nature. It may be that this medium inhibits their growth.

Three plates were always poured, and the mean of the three countings was taken for the result; in most cases fairly concordant numbers were obtained (see Table II*a*, which contains the results of a few enumerations taken at random).

* H. E. Durham. *Trans. Path. Soc.*, London, 1899, p. 262.

II. DISTRIBUTION OF B. COLI COMMUNE

A. HIGHLY POLLUTED WATERS

The highly polluted waters examined included several examples of crude sewage and of septic tank liquors, as well as of effluents from various sewage disposal works. In all these cases the bacillus was present in very large numbers, though filtration, if properly applied, seems to lessen the number very much.

The sewage at West Derby contained B. coli to the extent of 52,000 per c.c. on one occasion, and of 27,470 on another (see Table III). The sewage of Manchester showed a distinctly lower figure, 5,000 per c.c. being the mean of 5 determinations, while that of Leeds gave 19,700 as the mean of 6 determinations.

At Manchester the number of B. coli in the sewage was compared with the number in the septic tank liquors, both of the open septic tanks and of the Cameron. It was found that no increase took place, rather that there was a decrease. It must be noted, however, that the sewage had a distinct tarry odour, and probably contained many substances with antiseptic properties. The same thing was noticed in the case of the sewage and septic tank liquor at Leeds. Whether such a diminution would also take place in cases where the sewage is purely domestic is an interesting point. So far it would seem that the influence of the septic tank in sewage disposal was beneficial in reducing the numbers of B. coli.

The B. coli in sewage or in septic tank liquors can largely be removed by filtration. In the Dibdin system it is by no means eliminated (see Table III); the effluent from the experimental Dibdin beds at West Derby showed nearly 10,000 B. coli per c.c. A second filtration, which was continuous, improved the character of this effluent very much; the numbers decreased to 239 per c.c. when a small experimental sand filter was used, and to 89 per c.c. when earth was used as the filtering material. This latter figure was the average of 18 determinations, and often B. coli was absent altogether, the diminution depending largely on the rate of flow. This result is confirmed by the fact that a very small number of B. coli per c.c. was found in the effluents from the land drains of the West Derby Sewage Farm. Here the average from 7 different drains on January 22nd was only 50 B. coli per c.c.

Under the head of polluted waters one can consider rivers which receive much untreated sewage. The River Severn, at Shrewsbury, showed an average of 55 B. coli per c.c. on one occasion when the river was in flood; on a second occasion the average number per c.c. was 180. The River Alt, which is contaminated by sewage,

TABLE III

NUMBERS OF B. COLI COMMUNE PRESENT IN SEWAGE AND OTHER HIGHLY POLLUTED WATERS

Description	Date drawn	Analyzed	Dilution used	B. coli commune per c.c.	Remarks
West Derby Crude Sewage ...	19-10-99	21-10-99	·01	52,000	...
„ „	26-10-99	27-10-99	·005	27,470	...
Manchester Roscoe Beds, Effluent	5-12-99	6-12-99	·01	3,900	Mean from 3 different beds
„ Final Contact Bed Effluent ...	Mean of 3 analyses	in Dec., 99, and Ap., 00	·01	590	
„ Crude Sewage ...	„ 5 „	in Dec., 99	·01	5,000	These samples were plated the same day as they were drawn. Weather very cold, sewage, etc., smelling strongly of tar.
„ Cameron Tank Liquor ...	„ 4 „	„ Dec., 99	·01	2,100	
„ Open Septic Tank Liquor ...	„ 5 „	„ Dec., 99	·01	2,130	
Leeds Crude Sewage ...	Mean of 6 analyses	in Dec., 99, and Jan., 00	·01	19,700	Samples came by post and were plated a day after they were drawn.
„ Cameron Tank Liquor	„ 7 „	in Dec., 99, and Jan., 00	·01	6,500	
„ Septic Tank Liquor (No. 1 Tank) ...	„ 8 „	in Dec., 99, and Jan., 00	·01	4,060	
„ Septic Tank Liquor (No. 2 Tank) ...	„ 8 „	in Dec., 99, and Jan., 00	·01	4,300	
West Derby Sand Bed Effluent ...	Mean of 5 analyses	...	·01	9,300	...
„ Dibdin Bed Effluent ...	„ 19 „	...	·01	10,000	...
Above Dibdin Effluent, after subsequent filtration through small experimental filters.					
No. 1 Sand Filter ...	„ 17 „	...	Undiluted	240	...
No. 2 Earth Filter ...	„ 18 „	...	Undiluted	90	...
Land Effluents from West Derby Sewage Farm ...	From 7 different drains 22-1-00		Undiluted	50	...

HIGHLY POLLUTED RIVERS, ETC.

Manchester Ship Canal—					
Above sewer outfall ...	5-12-99	6-12-99	·01	6,100	...
At sewer outfall ...	„	„	·01	3,650	...
Below sewer outfall ...	„	„	·01	3,800	...
River Alt	22-1-00	22-1-00	·01	277	...
River Severn at Shrewsbury...	11-1-00	12-1-00	·1	55	River was in flood. Mean of 5 dets. at different places in the town.
„ „ „ ...	14-3-00	15-3-00	·1	190	Mean of 8 dets. at different places in the town.
„ „ „ ...	30-3-00	31-3-00	·1	180	Mean of 6 dets. at different places in the town.

showed B. coli to the number of 277 per c.c. The Manchester Ship Canal is not materially fouled by the addition of the Manchester sewage, containing, as it does, 6,100 B. coli per c.c. before the sewage enters it, and 3,800 afterwards.

B. NON-POLLUTED SUBSTANCES

The same method of plating with carbolic agar has also been found convenient for the examination of substances that are not obviously polluted. In such cases a very low dilution, or none at all, has been employed, and the plates contain for the most part colonies that are not B. coli; any colony that resembles B. coli, when looked at under the microscope, is investigated further by means of subcultures. As a rule, the distinction can be seen by merely observing the colony, and subcultures are made as a means of confirmation. The colony of B. coli is extremely typical on this medium, especially by its blue opalescence seen by transmitted light, and the oil-drop-like, colourless, appearance when viewed by reflected light. No colonies resemble those of B. coli exactly, though they may be a little like; in these cases a subcultivation on agar and coverslip preparation will often settle the point. As will be seen in cases where there has been no obvious pollution no B. coli is found, and in these cases the plates are often found to be quite sterile.

The substances examined were the following :—

(*a*) Air.
(*b*) Snow.
(*c*) Rain and hail.
(*d*) Dust.
(*e*) Virgin soil.
(*f*) Streams and rivulets.
(*g*) Sand of the sand filters of the Liverpool Waterworks.
(*h*) Filtered and other drinking waters.
(*i*) Grain and other food stuffs.

(*a.*) *Air.*—In the first experiments plates were poured and exposed to the air for 5, 15, 30 minutes. No B. coli were found, either in the air of the laboratory or in the air outside, by these means.

To make a more complete search for the presence of the bacillus the following plan was adopted :—

The air to be examined was drawn through a measured quantity of water in an Erlenmeyer flask, by means of a water pump, and measured by a gas meter. In the tube leading the air from the Erlenmeyer flask to the meter was a wool plug, and after the experiment was completed this plug was added to the water and well washed. Then 1 c.c. of this water was taken and plated. The air of the laboratory was analyzed in Experiments I and II.

Experiment I.—250 litres were drawn through in about 25 hours; three plates

were made of 1 c.c. of the 100 c.c. in the flask, and all found to be sterile, so that B. coli may be said to be absent in 7.5 litres. Also, 40 c.c. of the water was taken, 10 c.c. of (5 in 1,000) carbolized broth was added, and the mixture incubated for 24 hours at 42°, the proportion of carbolic acid in the mixture being equal to 1 in 1,000. After 24 hours there was slight turbidity in the broth, which when stroked on agar gave a growth which was unlike B. coli, and proved to be a micrococcus. By the phenol-broth method one can say that B. coli was absent in 100 litres ($\frac{40}{100}$ of 250 litres).

Experiment II.—A second experiment, in which 200 litres were drawn through at a rate of 10 litres per hour, gave a similar result. By plating on carbolic agar B. coli was found to be absent in 8 litres, and by incubation with carbolized broth in 80 litres.

Experiment III.—249 litres of the air outside were drawn through at a rate of about 11 litres per hour, and treated similarly. By carbolized agar plates, B. coli was found to be absent in 15 litres, and, by the phenol-broth method, absent in 199 litres.

In the next experiment the air was drawn through at a much greater rate, so that a great deal more was analyzed. It was found, in the previous experiments, that, of the cotton wool plug between the wash flask and the meter, the end nearest the wash flask became very dirty, showing that the water had but little effect in scrubbing the air, and hence in retaining the bacteria. The wash flask was therefore discarded and the air was passed through a plug of glass wool, which had been tightly packed in a glass tube and then sterilized. Glass wool was used instead of cotton wool, because the former can be so much more thoroughly washed. At the conclusion of the experiment the plug was pushed out, and the tube was washed down with sterile water into a sterile flask; 10 c.c. of water was used, and the whole thoroughly well shaken and then analyzed. On this occasion the air outside was examined, and in all 2,893 litres were drawn through in about 48 hours, from February 17th to February 19th, 1900. The water was incubated with carbolic broth, and also plated on carbolized agar; in neither case was B. coli found. By the first method it was found to be absent in 1,157 litres, 4 c.c. being used for analysis; and by the second absent in 867 litres. Adding the two results together, one may say that B. coli was absent in 2,025 litres. The weather at the time was very cold, and snow was on the ground. I am hoping to repeat the experiment in dry, dusty weather.

(*b*) *Snow.*—On February 10th, 1900, snow was collected off the ground in sterile petri dishes, brought into the laboratory and allowed to melt, ·5 c.c. and 1 c.c. being taken for analysis. Four plates were poured, and they were all found to be sterile.

(*c*) *Rain.*—On three occasions rain water was analyzed in order to see if the B. coli was present, but each time no B. coli was found. On January 17th, 1900, the first analyses of rain water were made; rain was collected in sterile petri dishes and 1 c.c. plated on carbol-agar. The plates, after incubation, were found to contain no B. coli. On the same day plates were poured and exposed to the rain with the same result.

On January 24th five samples of rain water were collected from gutters at different places on the roof of the laboratory. B. coli was found to be absent in .5 c.c. in each case.

The last analysis was made on April 3rd, 1900, three samples of rain were collected, as it was falling, in sterile petri dishes. In each case no B. coli was found in 1 c.c.

Hail was also analyzed with the same result. Unfortunately, the storm (January 27th, 1900) lasted a very short time, so that only a small quantity could be collected. The B. coli was, however, found to be absent in 1 c.c.

(*d*) *Dust.*—In all five samples of dust were examined; they were obtained on two occasions (February 27th and April 3rd) from different places in the laboratory, from the tops of cupboards, &c., where it was lying thick and undisturbed. The samples were taken by means of a sterile swab, 10 c.c. of sterile water was then poured into the test tube containing the swab and the whole thoroughly well shaken, and 1 c.c. of the mixture taken for analysis. This was the method adopted in the case of four samples, and no B. coli was found. In the case of the fifth sample, the swab was itself shaken successively in three tubes of melted carbolized agar, and these tubes then poured; these plates were also found to be free from B. coli, though by no means sterile. The plates had to be very carefully examined, and many subcultures made of colonies of which it could not definitely be said that they were not B. coli, although they had not the typical appearance. In every case these cultures proved to be an organism other than B. coli. However, for analyzing such substances as dust, earth, sand of filter beds, &c., it must be confessed that the method of plating with carbolized agar is by no means ideal, and requires a great deal of tedious work in examination of separate colonies before definite conclusions can be drawn as to the presence or absence of the B. coli.

On one occasion also the dust underneath the laboratory floor was collected and analyzed, one gram being weighed and shaken up with 20 c.c. sterile water. From this, typical B. coli were isolated, and they were present approximately to the extent of 10 per gram. The fact that the bacillus was found here and not in the dust lying upon the cupboards, etc., may probably be explained by the possibility of a culture of B. coli having at some time been dropped and broken on the floor, and may be traced to a direct infection.

(*e*) *Virgin Soil* (*moorland*).—Samples of moorland soil were obtained from different places on the high ground of the Lake Vyrnwy and Rivington watersheds. In all 15 samples were examined, and the details are given in Table IV. The analysis was made as follows: 1 gram of the soil was weighed into a sterile flask with the help of a sterile platinum spatula; 10 c.c. of sterile water were added in some cases, and 50 c.c. in others. The whole was then thoroughly well shaken and 1 c.c. taken for analysis, and, as usual, three plates of each were poured. The B. coli was found

to be absent in all 15 samples, in some absent in approximately .1 gram, in others in .02 gram.

(*f*) *Streams and Rivulets.*—The following experiments have reference to the water from sources which form the main Liverpool water supply. These are two in number ; Lake Vyrnwy acts as one reservoir, while the rest of the water of the city is taken from the Rivington Reservoirs. The water is filtered through sand filter beds at Oswestry and Rivington respectively, and the water supplied to the city consists of a mixture of the two supplies. A very complete analysis was made of the streams and rivulets of the watershed supplying Lake Vyrnwy. In the foregoing sets of analyses the material examined was obviously unpolluted, and B. coli was found absent in every case in the quantities analyzed ; of the previous history of these streams and rivulets one cannot be so sure. The water of thirty-eight streams was examined, 1 c.c. being taken for analysis in each case and three plates poured. The B. coli was found to be absent in thirty-one cases and its presence was demonstrated in seven. In these, however, it was present in very small quantities ; one organism per c.c. in one case, and less than this amount in the other six cases. The details of the sources, etc., of the water examined are given in Table V, and it is very interesting to notice that in nearly all the cases in which B. coli was found, there is very good evidence of the possibility of sewage pollution, and in many instances this had previously been suspected and the analyses were made with a view to clearing up this point.

It was found that, although on the whole the rivulets of the Vyrnwy watershed were free from the B. coli, yet that the sand of certain of the sand filters at Oswestry (see Table VI), used for the subsequent filtration of the Vyrnwy water, contained the bacillus to a considerable extent, so it was thought desirable to examine larger quantities of the water. About 2½ litres were concentrated by filtration through a Chamberland and Berkefeld Filter, the filter brushed with a measured quantity of water and 1 c.c. plated. These experiments have no great numerical accuracy, the object being rather to demonstrate the mere presence or absence of the colon bacillus. The surface water of Lake Vyrnwy, at Masonry Dam, was analyzed in this way two days after having been drawn. The water contained approximately 158 B. coli per litre. The water of the Cedig River, analyzed four days after having been drawn, showed 133 per litre. Probably these figures are rather lower than they should be, as the B. coli tends to decrease in numbers when kept in fairly pure water in a closed bottle. This has been pointed out previously in this paper and is further confirmed by the following experiment. Water from the surface of the lake near the Tower was examined 25 days after having been taken ; B. coli was found to be absent in at least 514 c.c. Unfortunately the same water was not analyzed at the time it was drawn, or shortly afterwards ; but it is probable it would then have contained the B. coli, though probably not in 1 c.c.

The rivulets of the watershed of the Lower Rivington Reservoir were

similarly examined. The plates of the analyses of January 17th were full of colonies, contrasting with those made from the Vyrnwy waters, which were, in many cases completely sterile. On this occasion the B. coli, when present, was found in greater numbers, about 10 per c.c. in some cases, though definite enumerations were not made. On the other occasions the numbers of B. coli when found were as low as was the case in the Vyrnwy waters. Fifty-three samples were analyzed; B. coli was found to be absent in thirty-eight cases, and present in eleven cases; in four cases its presence was doubtful (see Table V*a*). Here, as was the case with the rivulets of the Vyrnwy watershed, many of the streams shown to contain B. coli have long been suspected of pollution, and in the other cases there is distinct evidence of the possibility. The water of the Rivington Reservoir was also examined in quantity, about 2½ litres being used for analysis. Water from the surface of the reservoir showed the B. coli to be absent in 119 c.c., while water from the depth contained the bacillus to the extent of 27 organisms per litre (approx.). These analyses were made one and three days respectively after the samples had been taken.

The streams and drains flowing into the River Severn near Shrewsbury were also analyzed (see Table V*b*). Seventeen analyses were made, and in twelve cases B. coli was absent in 1 c.c.; in the others it was present to a greater or less degree. Number 11, which contains B. coli commune to the extent of 19 per c.c., enters the River just below Wroxeter, and, from the map, it seems probable that it would be contaminated with domestic sewage. Number 15 is a stream which receives the sewage from Atcham Union Workhouse. There are considerable difficulties in the way of finding out exactly the previous history of such streams and drains as these; probably all those containing B. coli have received some definite pollution. The fact that the majority of the land drains are free from B. coli is, I believe, contrary to a statement that is often made, that this bacillus is to be found in quantity in all cultivated lands, and hence in their drainage. Of course, such water has been subjected to a very fair filtration before it enters the drains.

(*g*) *Sand of Filters used for filtering Drinking Water.*—The water of the Vyrnwy Lake is filtered through sand beds before being used for drinking purposes. It contains the Bacterium coli commune, though to a very small extent. All attempts to detect the presence of the bacillus in the filtered water have failed, even when large quantities have been used for analysis (see below). Hence it seemed probable that the sand beds used for filtering might retain the bacillus, and this has proved to be the case, confirming other experiments on the efficiency of sand as a medium for filtration. The sand of the filter beds was analyzed in the same way as the soils previously described; in these cases a dilution of 1 gram in 10 c.c. of sterile water was invariably used. The details are given in Table VI. Out of 15 samples analyzed, B. coli was absent in .1 gram in 8 cases and present in 7. In one of these cases

the approximate number of B. coli per gram of sand was as high as 2,055, while in another place on the same bed it was 20.

Similar analyses were made of the sand of the filters at Rivington, which filter the water of the Rivington Reservoir. The sand of three of the beds was examined; in all 14 analyses were made, and the B. coli was found to be absent in each case (see Table VI*a*.)

(*b*) *Drinking Water.*—Eighteen samples of filtered water from the filters at Rivington were examined, and in every case B. coli was found to be absent in 1 c.c. (see Table VII). The tap water of the laboratory was also found to contain no B. coli in 1 c.c. This water was also analyzed in quantity on two occasions (March 9th and March 13th, 1900), about 2½ litres being concentrated by means of a Chamberland and Berkefeld Filter respectively, and a portion of the concentrated water used for analysis. In the one case B. coli was found to be absent in 900 c.c., and in the other in 823 c.c. Of course, the Liverpool water supply is unusually excellent.

On February 26th the dead ends of the pipes supplying certain streets in Liverpool were cleaned out by flushing with water, and samples were taken at certain intervals of the dirty-looking water which came out (see Table VII*a*). Sixteen samples were taken and analyzed, and B. coli was found to be absent in every case.

The drinking water of Shrewsbury was analyzed (November 18th, 1899) and showed the presence of B. coli. The water supplied to the town by conduits from a distant source contained 2 B. coli per c.c. The river water, filtered, is also largely used in the town, and contained 17 B. coli per c.c.; this water is of an undeniably polluted origin. The water of the river, at the point where the town supply is drawn off, has on several occasions been analyzed and has always contained the B. coli.

(*i*) *Grain and other Foodstuffs.*—These analyses were made in order to institute a comparison between the respective distribution of B. coli commune and B. enteritidis sporogenes. The duplicate analyses were made by Dr. Balfour Stewart, who also investigated the significance and comparative frequency of the latter organism (see this volume, pp. 31 and 34). In all 19 samples of grain were examined, including English and foreign wheat, both before and after malting, rice, oats, &c. The analyses were made thus:—Different quantities of the grain were placed in a sterile flask, and well washed with 10 c.c. sterile water, 1 c.c. of this being used for plating. B. coli commune was found to be absent in every case (see Table IX). Some samples of crushed grain were also examined; no particular quantities were taken, a little was well shaken up with 10 c.c. sterile water, and 1 c.c. plated as before. B. coli was absent in crushed malt, oat husks, bran, and also in a sample of flour, which was the coarsest quality sold.

The comparative infrequency of the occurrence of B. coli commune in foods may also be seen from Table IX, which contains a summary of food analyses made in the Thompson Yates Laboratories for the Liverpool Corporation, extending over

a period of about six months. It will be seen that out of a total of 440 foods analyzed B. coli commune was present only in 19 cases; 17 of these referred to milk, where its presence could easily be explained by contamination occurring in the cowsheds. The remaining two cases were those of mussels, very possibly procured from an estuary, where pollution by sewage is a common occurrence.

TABLE IV

SAMPLES OF SOIL FROM MOORLAND

Sample Number	Date	Source	B. coli
1	Jan. 27th	Bryn Gwyn Hill	Absent in .02 gram.
2	" "	" " "	" "
3	" "	" " "	" "
4	" "	Fawnog, at back of Tyn-y-Garry, N.E. Side of Lake	" "
5	" "		" "
6	" "		" "
7	Feb. 6th	S. side of Hirddu River ...	Absent in .1 gram.
8	" "	W. side of Eunant River ...	" "
9	" "	S. side of Iddew River	" "
10	" "	N.E. side of Nadroedd River ...	" "
11	" "	N.E. side of Garneddwen Stream...	Absent in .1 gram.
12	" "	W. side of Cedig River... ...	" "
	March 17th	From that portion of the Rivington watershed which drains into the Yarrow, or 2nd Rivington Reservoir	
13	" "	1. A small watercourse running through flat boggy ground ...	Absent in .1 gram.
14	" "	2. From steep bank of stream which falls rapidly over a rocky course near the boundary of the watershed	Absent in .1 gram.
15	" "	3. From the top slope of Rivington Pike, a steep bank of exposed soil which drains into River Douglas	Absent in .1 gram.

TABLE V

Streams of the Lake Vyrnwy Watershed

No. of sample bottle	Date	Source	B. coli commune	Remarks
49	Jan. 11, 1900	Llettyreos Stream ...	Less than 1 per c.c.	
9	„ „	Nant-y-Maes Stream ...	„ „	
51	„ „	Pistill-y-Ceunant Stream...	Absent	
59	„ „	Llwynrhiw Stream ...	Less than 1 per c.c.	Very possible contamination from a farm-house on the margin of the stream ; a suspected case
135	„ „	Hirddu River... ...	Absent	
45	„ „	Stream S.E. of Eunant Quarry	Bottle broken	
34	„ „	Stream N.W. of Eunant Quarry	Absent	
32	„ „	Eunant River	Absent	
13	„ „	Nant Llwyn	1 per c.c.	
143	„ „	Nadweddor Rhiwargor River	Less than 1 per c.c.	There are dwelling houses near, but their drainage has been carefully attended to ; no apparent cause for suspicion
140	„ „	Iddew or Blaen-y-Coed River	„ „	„ „
35	„ „	Alltforgan Stream (taken near house) ...	Absent	
44	„ „	Ceunant Moel-y-Nant Stream	„	
55	„ „	Garneddwen Stream ...	„	
56	„ „	Ceunant-y-Ffinnant ...	„	
5	„ „	Llechwedd	„	
11	„ „	Cedig River	„	
17	„ „	Surface of Lake at Tower	„	
72	„ „	Stream W. of Hendre ...	„	
33	„ „	Stream N. of Hendre ...	„	
67	„ „	Compensation water drawn from near bottom of Lake	„	
96	Jan. 22, 1900	Stream Glyn-du ...	„	
48	„ „	Stream N.W. of old Nursery	Present Less than 1 per c.c.	There was a gang of men working close to this stream at this date

TABLE V—*continued*

No. of sample bottle	Date	Source	B. coli commune	Remarks
25	Jan. 22, 1900	Stream N.W. of Shanty ..	Absent	
64	„ „	Stream N.W. of new Nursery	„	
50	„ „	Stream between Nant-y-Maes and Pistill-y-Ceunant	„	
28	„ „	Stream passing through Llwynrhiw House (above house) ...	„	
26	„ „	Stream N.W. of Craig-y-Gribin	„	
53	„ „	Stream 180 yards S.E. of Eunant Quarry ...	„	
65	„ „	Stream 100 yards S.E. of Eunant Quarry ...	„	
71	„ „	Stream N.W. of Fyn-y-Coed	„	
132	„ „	Stream 190 yards N.W. of Eunant Quarry ...	„	
57	„ „	Stream S.E. of Garneddwen	„	
1	„ „	Stream by Gwyddaran and Ty-mawr or Ffynnon Dogfan	„	
8	„ „	Stream by Gwyddaran or 4 miles 4 chains ...	„	
22	„ „	Stream N.W. of Llechwedd	„	
42	„ „	Stream S.E. of Llechwedd	„	
46	„ „	Stream between Cedig Bridge and Glanvyrnwy	..	
18	„ „	Stream S.E. of Vrongoch	..	

TABLE V*a*

STREAMS OF THE WATERSHED OF THE LOWER RIVINGTON RESERVOIR

Date	Source	B. coli commune in 1 c.c.	Remarks
Jan. 17, 1900	River Douglas, opposite flood stop planks	Absent	*The carbolized agar plates of this date were so full of colonies that in some cases, where B. coli is stated to be absent it is doubtful whether there may not have been present one or two very small colonies. In cases where it was present, it was frequently found to the extent of 10 per c.c.
,, ,,	River Douglas, just below stream through Jephson's Farmyard	Absent	
,, ,,	Stream into Douglas diversion, just above ornamental water	Absent	
,, ,,	Stream into head of Ettock's Creek...	Absent	
,, ,,	Stream into Ryder's Creek from Ward Hill Farm... ...	Doubtful	There is a house close to the stream
,, ,,	Stream between Top-of-Hill Farm and Tanpits	Present	Pollution from farmyard drainage has been suspected
,, ,,	Stream into Taylor's Creek below Great House Farm ...	Absent	
,, ,,	Stream below School Brow Farm	Present	A suspected case of pollution
,, ,,	School Brook	Present	Vicarage and cottages close to the stream; a suspected case of pollution
,, ,,	Ditch below 'Black-a-Moor's' Cesspool, inside Reservoir...	Doubtful	A suspected case of pollution
,, ,,	Iron outlet pipe inside Reservoir walls	Absent	
,, ,,	Outlet of stone drain a few yards to the S. of last	Absent	
,, ,,	Stream below Anderton Hall Stables	Absent	
,, ,,	Drain below Anderton Hall Stables	Present	A suspected case of pollution
,, ,,	Land drain in bend of Reservoir	Absent	
,, ,,	Similar outlet in next bend to south	Doubtful	No suspicion
,, ,,	Outlet of iron drain below Anderton Hall Cesspool ...	Present	
,, ,,	Stream, N. boundary of ornamental grounds, Anderton Hall	Absent	
,, ,,	Outfall drain at Reservoir gate, Anderton Hall	Absent	
,, ,,	Outfall of land drains below Tanpits Farm	Absent	
,, ,,	Drain which receives overflow from pond in yard of Tanpits Farm	Present	Has been suspected of receiving Farmyard drainage

TABLE V*a*—*continued*

Sample bottle No.	Date	Source	B. coli commune in 1 c.c.	Remarks
110	* Jan. 29, 1900	River Yarrow at Corporation boundary	Absent	*The carbolized agar plates were not at all overgrown on this occasion, and contained on an average less than 10 colonies on a plate, and certainly no B. coli in cases in which it is stated to be absent
120	„ „	Trough on Farm Road to Parson Bullvagh's Farm	Absent	
62	„ „	Outlet end of tunnel from Dean Wood to Yarrow Reservoir ...	Absent	
135	„ „	Water supply to Anderton's Farm, Yarrow Reservoir	Absent	
128	„ „	Trough in yard of 'Black-a-Moor's Head' Hotel	Absent	
118	„ „	Public drinking trough, Rivington Village	Absent	
	„ „	Drinking trough at Haddock's Fold, Anglezark	Absent	
132	„ „	Stream from Yarrow Reservoir ...	Absent	
7	„ „	Drinking trough at Brook House, Anglezark	Absent	
89	„ „	Stream at or from Chorley Reservoir	Absent	
126	Jan. 31, 1900	Farm trough on bank of Dean's Reservoir (west side) ...	Absent	
73	„ „	Stream flowing into Dean's Reservoir from Peter's Farm ...	Absent	
82	„ „	Small Stream which joins last before flowing into Dean's Reservoir...	Absent	
69	„ „	Well, behind Royal Farm, on Darwen watershed	Absent	
28	„ „	Stream flowing into Upper Roddlesworth Reservoir, at outlet end of culvert under road, Hollingshead Mill	Absent	
86	„ „	Stream below Hollingshead Terrace, flows into Upper Roddlesworth	Absent	
22	„ „	Drinking water supply Halliwell Fold Farm	Present, 1 per c.c.	Suspected pollution from farmstead
93	„ „	Stream flowing down Farm Road from Halliwell Fold into River Roddlesworth	Present, 1 per c.c.	„ „ „
129	„ „	River Roddlesworth, Bridge from Halliwell Fold	Present, 1 per c.c.	„ „ „
0	Feb. 1, 1900	Drain emptying into Lower Rivington Reservoir	Absent	
61	„ „	Outlet drain emptying into Lower Rivington Reservoir, being overflow from water supply to Tanpits Farm	Absent	

TABLE V*a*—*continued*

Sample bottle No.	Date	Source	B. coli commune in 1 c.c.	Total Nos. of bacteria per c.c.	Remarks
18	* Feb. 23, 1900	Stream by roadside, Lower Roddlesworth Farm	Absent	—	*All the samples of this date were suspected cases with the exception of No. 34
34	,, ,,	Small Spring on slope of field in front of Aushaw's Farm ...	Absent	184	
132	,, ,,	Stream below Well in same field as last...	Absent	312	
22	,, ,,	Overflow from Well in middle of field between Deal Fold Farm and Rake Brook... ...	Present, 1 per c.c.	1005	Presence of B. coli agrees with a large increase in total numbers
43	,, ,,	Overflow from Well below Snape's Farm	Absent	56	
122	,, ,,	Small Stream down hedgeside from Botany Bay Farm to Rake Brook	Present, 1 per c.c.	Plate liquefied.	Presence of B. coli corresponds with increase in total numbers
48	,, ,,	From Rake Brook, below inflow of last Stream	Absent	392	
37	,, ,,	Water lying in grip on field in front of Botany Bay ...	Doubtful (prob. absent)	1184	
127	,, ,,	Stream by roadside issuing from rock, Norcross Farm ...	Absent		
143	,, ,,	Standpipe at No. 2 Bridge ...	Absent		
118	,, ,,	From Goit below No. 2 Bridge...	Absent		

TABLE V*b*

RIVULETS, DRAINS, ETC., DRAINING INTO THE RIVER SEVERN IN THE NEIGHBOURHOOD OF SHREWSBURY

No.	Date	Source	B. coli commune per c.c.
1	Mar. 14, 1900	Land Drain, L. bank above Penley Rough	Absent
2	,, ,,	Land Drain, R. bank opposite Penley Rough	Absent
3	,, ,,	Land Drain, above Uffington, L. bank	Absent
4	,, ,,	Mill Brook, Uffington, L. bank ...	16
5	,, ,,	Land Drain, R. bank, Ford ...	Absent
6	,, ,,	Land Drain	Absent
7	,, ,,	Land Drain, Railway Bridge ...	Absent
8	,, ,,	Land Drain, at Municipal Boundary, below Preston ...	25
9	,, ,,	Land Drain, below R.	Absent
10	,, ,,	Land Drain, Bell Brook ...	Absent
11	,, ,,	Land Drain, below Wroxeter ...	19
12	,, ,,	Cound Brook	1
13	Mar. 30, 1900	Land Drain, L. bank	Absent
14	,, ,,	Land Drain, Burton's	Absent
15	,, ,,	Drain, from Atcham Union Workhouse	19,000
16	,, ,,	Land Drain, Coton's	Absent
17	,, ,,	Land Drain, R. Bank, Lower Cound Farm	Absent

TABLE VI

Sand Filters at Oswestry

Number of Sample Bottle	Date	Source	B. coli commune Approx. per grm.
33	Jan. 26, 1900	Surface of Test Filters (No. 6) ...	10
43	„ „	6″ Pipe Filters (C)	Absent.
		Taken from three different places	
44	„ „	No. 1 Large Filter...	38
72	„ „	„ „	57
143	„ „	„ „	73
43	Feb. 10, 1900	No. 2 Filter	Absent in .1 grm.
33	„ „	No. 2 Filter (a different place) ...	3
127	„ „	No. 4 Filter	Absent in .1 grm.
120	„ „	No. 4 Filter (a different place) ...	Absent in .1 grm.
114	„ „	No. 5 Filter	2055
40	„ „	No. 5 Filter (a different place) ...	.20
143	„ „	No. 7 Filter	Absent in .1 grm.
122	„ „	No. 7 Filter (a different place) ...	Absent in .1 grm.
118	„ „	No. 8 Filter	Absent in .1 grm.
18	„ „	No. 8 Filter (a different place) ...	Absent

TABLE VI*a*

Filters at Rivington

Number of Sample Bottle	Date	Source	B. coli commune
5	Jan. 29, 1900	From surface of No. 6 Filter. Taken with full head of water on bed. This bed has been worked up for 60 days since last cleaning	Absent in .02 grm.
141	" "	Same as last	Absent in .1 grm.
68	" "	From surface of No. 7 Filter. Taken after head of water had been reduced to 1 foot. This bed has been in work for 38 days since last cleaning ...	Absent in .02 grm.
7	" "	Same as last	Absent in .1 grm.
57	Feb. 24, 1900	Surface of Filter No. 7, bed in use since Dec. 22nd, 1899 ...	" "
134	" "	" " " ...	" "
57	" "	" " " ...	" "
134	" "	" " " ...	" "
65	Mar. 10, 1900	Six different Places in No. 5 Filter Bed, along centre line equally distant from each other	" "
64	" "		" "
115	" "		" "
17	" "		" "
1	" "		" "
137	" "		" "

TABLE VII

Filtered Waters, Rivington

Analyzed March 5th, 1900

Bottle No.	Description of Sample	B. coli commune in 1 c.c.
118	No. 8 Filter, working since January 1st	Absent
122	Well Hole	,,
31	Clear Water Tank No. 2	,,
43	Feeder No. 2...	,,
22	No. 1 Filter, working since December 23rd, 1899 ...	,,
33	Well Hole	,,
121	Clear Water Tank No. 1	,,
80	No. 1, Filter working since January 3rd	,,
19	Water Hole	,,
140	No. 3, Filter working since January 6th	,,
135	Well Hole	,,
49	No 4, Filter working since December 11th, 1900 ...	,,
132	Well Hole	,,
48	No. 5, Filter working since December 9th, 1899 ...	,,
47	Well Hole	,,
127	No. 6, Filter working since December 1st, 1899 ...	,,
114	Well Hole	,,
34	Feeder No. 1	,,

TABLE VII*a*

Dead Ends

Date	Place	Time	B. coli commune in 1 c.c.
Feb. 26, 1900	Pembroke Place ... ... Ashton Street, Liverpool	1st minute ...	Absent
		2nd „	„
		3rd „	„
		5th „	„
		6th „	„
„ „	Prinfield Street, and of Chatham Place, Liverpool	1st minute ...	Absent
		2nd „ ...	„
		3rd „ ...	„
		4th „ ...	„
		5th „ ...	„
		6th „ ...	„
„ „	Smithdown Road, Liverpool	1st minute ...	Absent
		2nd „	„
		3rd „	„
		4th „	„
		5th „	„

TABLE VIII

GRAINS

Description	Quantities used for Analysis		B. coli commune
	Sterile water	No. of grains	
Wheat (English)...	10 c.c.	3	Absent
Wheat (Foreign)	10 c.c.	3	Absent
Barley	10 c.c.	3	Absent
Barley (Malted)	10 c.c.	3	Absent
Barley (Maited and Crushed) ...	10 c.c.	—	Absent
Rice (Rangoon)	10 c.c.	5	Absent
Rice (Java)	10 c.c.	24	Absent
Peas (English)	10 c.c.	1	Absent
Peas (Wisconsin)	10 c.c.	4	Absent
Peas	10 c.c.	4	Absent
Haricots (Foreign)	10 c.c.	4	Absent
Green Lentils (Whole)	10 c.c.	12	Absent
Rye	10 c.c.	24	Absent
Oats	10 c.c	24	Absent
Oat Husks	10 c.c.	—	Absent
Bran	10 c.c.	—	Absent
Flour (very Coarse)	10 c.c.	—	Absent
Hemp	10 c.c.	12	Absent
Linseed	10 c.c.	24	Absent

TABLE IX

LABORATORY ANALYSES OF FOODS, NOV. 11TH, 1899, TO APRIL 30TH, 1900

(See also this volume, p. 37)

Description of Food	No. of samples examined	No. of samples in which B.coli commune was present
Drinking Water	53	0
Milk (New)	239	17
Milk (Skim)	3	0
Milk (Condensed)	10	0
Butter	12	0
Cheese	4	0
Margarine...	4	0
Oatmeal and Flour	11	0
Tinned Meat, Fish, &c.	22	0
Shell-fish (Fresh)	66	2
Sausages	1	0
Fluid Beef	1	0
Suet	1	0
Worcester Sauce, &c.	2	0
Preserved Fruits and Jams	11	0
TOTAL	440	19

ON THE DISTRIBUTION OF BACILLUS ENTERITIDIS SPOROGENES

By C. BALFOUR STEWART, M.A., M.B. Camb., Assistant Bacteriologist to the Liverpool Corporation

Plate II

In connection with some fish poisoning cases which occurred in Liverpool last September, where death was due to enteritis associated with the presence of Bacillus enteritidis sporogenes of Klein, I had occasion to examine samples of fish procured from the same stores that supplied the fish which was incriminated in these cases, and from that I was led to examine other substances with a view to discover, if possible, the source from which the germ is distributed in nature.

That the germ occurs very widely spread has been observed by its discoverer, Klein, and others; but so far it has been looked for and found chiefly in what may be called pathogenic substances, as diarrhoeal stools, horse dung, sewage, etc., and in milk, where its presence was taken as denoting contamination.

The object of this paper is to draw attention to the fact that Klein's Bacillus enteritidis sporogenes occurs very widely distributed in quite innocent substances. Any remarks I may have to make on the cultural characteristics of the bacillus will be reserved for a future occasion; but I may here mention that I have found it by no means so easy to obtain a pure culture of the bacillus on solid media as Klein states in his paper in the Local Government Board Report for 1897-8. The reason may be that, from the nature of my samples, there were many other spore-bearing micro-organisms present which ran through the milk cultures and appeared on the glucose agar tubes inoculated from the milk; and even, in some cases, appeared in solid media cultures inoculated from the exudation of an animal killed by an injection of the milk culture. Often no growth at all on solid media was obtained from the exudation of a dead animal or from a milk culture, although a milk tube inoculated with the same grew and showed the typical reaction. Milk is a particularly good medium for the growth of the Bacillus enteritidis; whereas, as is well known, anaerobes in general do not grow well on solid media. It was found, by experiment, that if a milk tube was inoculated with B. enteritidis and some other micro-organism, as B. mesentericus, B. cadaveris (Klein), B. coli, the tube would show the typical reaction of B. enteritidis sporogenes after incubation for 18 hours.

In all these experiments litmus milk tubes were inoculated with a small portion of the sample; in the case of the milk which was done in the ordinary course of analysis for the Liverpool Corporation about 10 c.c. was placed in a sterile test tube.

The litmus milk, or plain milk tubes, were then placed in an apparatus described by me in another part of these Reports, where they were kept automatically at a temperature between 75° C. and 80° C. for 20 minutes. They were afterwards placed in wide mouth stoppered bottles, into which the requisite amount of pyrogallic acid and caustic potash was poured to insure anaerobiosis ; the bottles were then placed in the incubator at 37° C.

Usually all the tubes that 'went' typically had 'gone' after 18 hours' incubation. By 'typical' I mean the reaction described by KLEIN : the milk is clotted, and the clot turned into clear whey with an acid reaction, a broken or scattered layer of cream remaining on the top broken up by the gas which may be seen evolved in bubbles from the whey. If litmus milk was used, the whey and cream which was bleached in the anaerobic bottle will be seen gradually to assume a pink colour on being exposed to the air, instead of its original violet colour ; this is due to the altered reaction. The tube generally smells more or less of butyric acid.

With many of my samples the butyric acid smell was masked by a putrid smell ; sometimes both could be distinguished ; this occurred chiefly in the samples of grains, and was probably due to the presence of some other spore-bearing micro-organism causing putrefactive changes. On making one or two subcultures in milk, or a milk culture from the exudation of an animal killed by injection of a culture with a putrid smell, the smell would often be found to have disappeared and the milk culture to be in every respect typical. A certain proportion of tubes 'went' in the ways KLEIN describes as atypical and intermediate. An atypical tube is one devoid of gas formation, and, consequently, the layer of cream remains unbroken ; in the intermediate type the clot is not fully peptonised into whey, the tube contains one to two-thirds excavated clot and the rest is whey, there is also less gas formation.

With the following samples whenever a tube 'went' typically, atypically, or in the intermediate way, a portion of the whey was inoculated into Guinea-pigs beneath the skin over the abdomen ; 2 c.c. of the whey were inoculated in most cases, and, as the Guinea-pigs were all fully grown, this dose would correspond to KLEIN's dose for standard virulence, namely, 1 c.c. per 200-300 grams of Guinea-pig. According to KLEIN, the pathogenicity to Guinea-pigs is the means of distinguishing his Bacillus enteritidis sporogenes from BOTKIN's butyric acid bacillus, which gives the same reaction in milk cultures, but is non-pathogenic. As a result of subcutaneous inoculation, if the culture is virulent, the Guinea-pig will be found dead in 18 to 36 hours. On examination it will be found that the skin is separated from the subjacent tissues over a considerable area by dissolution of the connective tissue ; the skin may have sloughed through to the outside, or the muscles of the abdominal wall may have sloughed into the abdominal cavity. The space between the skin and the abdominal and thoracic walls contains more or less badly-smelling sanguineous fluid, but the fluid may be very scanty or almost absent in the most rapidly fatal cases.

If the culture is less virulent the post-mortem appearances will be modified, the separation of skin will be over a less extensive area, and if the culture is very little pathogenic there may only be a slight separation of skin with only a small quantity of fluid in the space. In these cases the fluid is gradually absorbed and the separated skin dries, and after one to two weeks sloughs off, leaving an ulcer. In a few cases the whey is quite non-pathogenic ; it is absorbed, and the animal shows no signs of being ill.

1. *Dried Salted Codfish.*—A litmus milk tube was inoculated with a small shred ; after 48 hours' incubation it had 'gone' typically. 2 c.c. whey were inoculated into a Guinea-pig. Result—Died in 18 hours.

2. *Dried Salted Haddock.*—A litmus milk tube was inoculated with a small shred ; after 18 hours it had 'gone' typically. 2 c.c. whey were inoculated into a Guinea-pig. Result—It was found dead in 16 hours, the skin over the abdomen and thorax was completely separated from the subjacent muscular tissue and there was a considerable quantity of badly-smelling sanguineous fluid in the space.

3. *Boiled Cod and Potatoes.*—Litmus milk tubes were inoculated with small portions of the fish and of the potato with negative results for B. enteritidis sporogenes.

4. *Salted Ling.*—A litmus milk tube was inoculated with negative results for B. enteritidis sporogenes.

5. *Salted Cod.*—A litmus milk tube was inoculated with a small shred ; after 18 hours' incubation it had 'gone,' but the whey was slightly yellow and had a putrid smell, a subculture of this in litmus milk 'went' and smelt of butyric acid. A Guinea-pig was inoculated with 1 c.c. of the whey from the first tube. Result—Non-pathogenic.

6. *Salted Haddock.*—A litmus milk tube was inoculated with a small shred with negative result for B. enteritidis sporogenes.

7. *Salted Cod.*—A litmus milk tube was inoculated with a small shred with negative result for B. enteritidis sporogenes.

8. *Salted Cod.*—A litmus milk tube was inoculated with a small shred from a clean part of the fish with negative results for B. enteritidis sporogenes. Another tube was inoculated with a shred from a spot where there was a mark as if from a dirty finger ; after 18 hours' incubation this tube had 'gone' typically. A Guinea-pig was inoculated with 1 c.c. of the whey. Result—Died in 30 hours and had separation of the skin and red fluid in the space.

9. *Salted Cod.*—A litmus milk tube was inoculated with a small shred. There was little change after 18 hours' incubation, but after some days it had 'gone.' 2½ c.c. whey were inoculated into a Guinea-pig. Result—Died in about 40 hours with complete separation of skin over thorax and abdomen.

10. *Salted Cod.*—A litmus milk tube was inoculated with a small shred ; after 18 hours incubation it had 'gone' atypically and had a putrid smell. A Guinea-pig was inoculated with 2 c.c. whey. Result—Died in 48 hours with considerable separation of skin and sloughing of abdominal muscle.

11. *Salted Cod.*—A litmus milk tube was inoculated with a small shred with negative results for B. enteritidis sporogenes.

12. *Salted Haddock.*—A litmus milk tube was inoculated with a small shred ; after 18 hours' incubation it had 'gone' typically and well, the cream being scattered. A Guinea-pig was inoculated with 2 c.c. Result—Pathogenic but not fatal.

13. *Salted Cod.*—A litmus milk tube was inoculated with a small shred with negative results for B. enteritidis sporogenes.

14. *Salted Haddock.*—A litmus milk tube was inoculated with a small shred; after several days incubation it had 'gone.' A Guinea-pig was inoculated with 2½ c.c. whey. Result—Non-pathogenic.

15. *Salted Ling.*—A litmus milk tube was inoculated with a small shred with negative results for B. enteritidis sporogenes.

16. *Salted Cod.*—A litmus milk tube was inoculated with a small shred; after several days' incubation it had 'gone.' 3 c.c. whey were inoculated into a Guinea-pig. Result—Non-pathogenic.

Thus, in 16 samples of fish 6 gave cultures of B. enteritidis of which 5 were fatal when inoculated into Guinea-pigs.

Grains taken from Sample Boxes in a Saleroom of a Wholesale Firm

(See Plate II)

Inoculated into milk incubated anaerobically 18 hours at 37° C.

1. *English Wheat.*—Milk 'went' in intermediate way; putrid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours; extensive separation of skin; very little exudation.

2. *Foreign Wheat.*—Milk 'went' typically; cream scattered; slight putrid smell. Guinea-pig inoculated with 2 c.c. whey. Pathogenic but not fatal.

3. *Bran.*—Milk 'went' well; cream raised to the cotton plug. Guinea-pig inoculated with 2 c.c. whey. Died in 18 hours.

4. *Oats.*—Milk 'went' slowly and not typically. Guinea-pig inoculated with 2 c.c. whey. Pathogenic but not fatal.

5. *Ground Oats.*—Milk 'went' typically. Guinea-pig inoculated with 2 c.c. whey; died 36 hours; much separation of skin; little or no fluid.

6. *Oat Husks.*—Milk after re-incubation had clotted; there was very little whey and no gas. Guinea-pig inoculated with 1 c.c. whey; non-pathogenic. Not B. enteritidis.

7. *Barley.*—Milk 'went' well; cream raised to the cotton plug. Guinea-pig inoculated with 2 c.c. whey; sloughing, ulcer. Pathogenic but not fatal.

8. *Malt, i.e., Kilned Barley.*—Milk 'went' only after re-incubation, and then not well. Guinea-pig inoculated with 2 c.c. whey; ulcer. Pathogenic but not fatal.

9. *Crushed Malt.*—Milk did not 'go'; a little whey formed, rest was clot; putrid smell. Guinea-pig inoculated with 1 c.c. whey; non-pathogenic. Not B. enteritidis.

10. *Rye.*—Milk did not 'go' till after re-incubation, when a yellowish whey formed with a putrid smell. Guinea-pig inoculated with 2 c.c. whey; died in 36 hours with much separation of tissues.

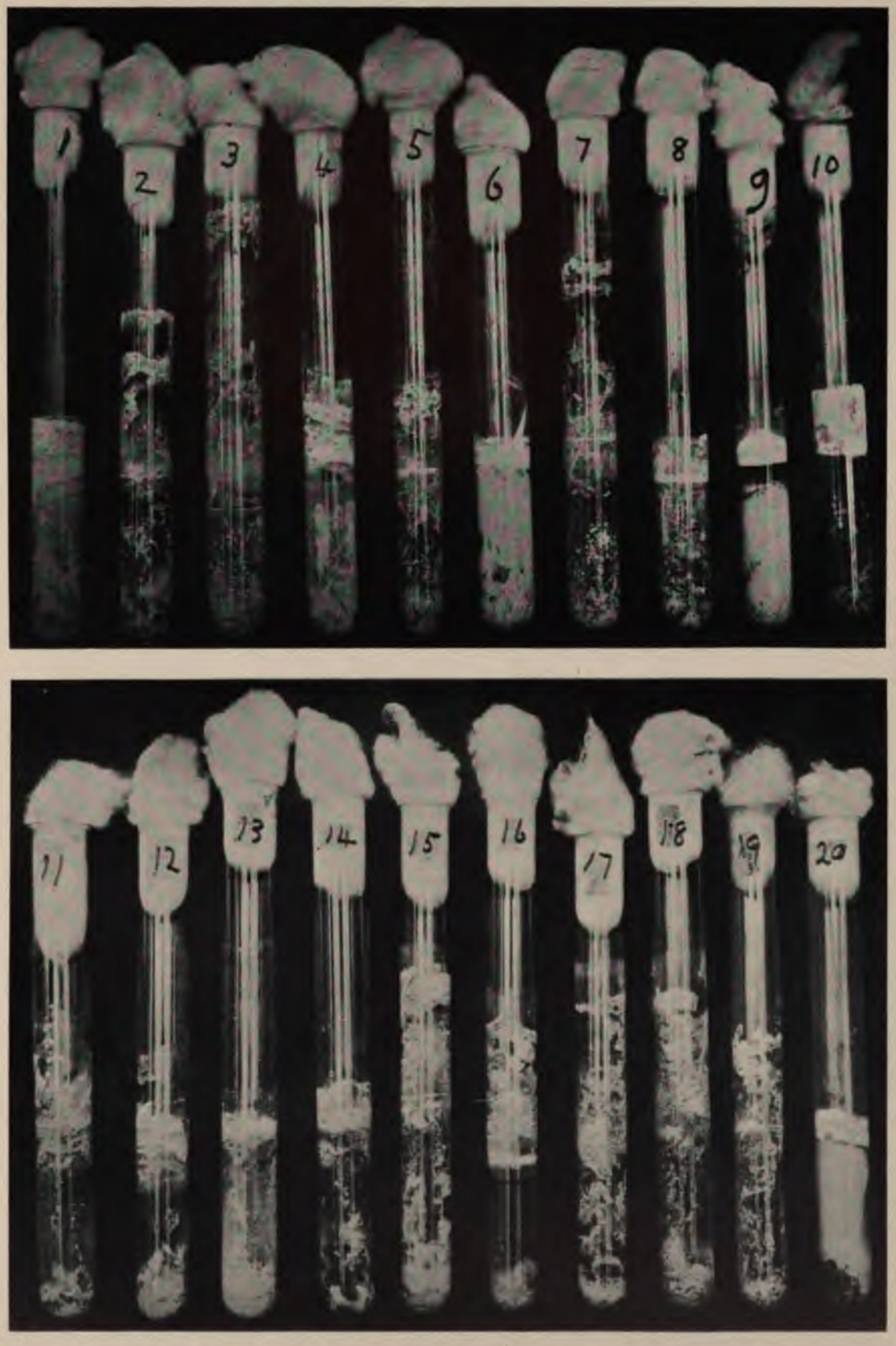
11. *Maize.*—Milk 'went' typically. Guinea-pig inoculated with 2 c.c. whey; died in 36 hours with separation of skin and sanguineous fluid in the space.

12. *Rice (Java).*—Milk 'went'; the whey had slightly putrid smell. Guinea-pig inoculated with 2 c.c. whey; died in 36 hours with separation of skin and red fluid in the space.

13. *Rice (Rangoon).*—Milk 'went'; whey had butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with very extensive separation of skin. There was little or no fluid in the space.

14. *Lentils.*—Milk 'went'; there was a little yellowish whey with a butyric acid and putrid smell. The cream layer was not raised. Guinea-pig inoculated with 2 c.c. whey; ulcer slightly pathogenic.

15. *Haricot Beans (Foreign).*—Milk 'went' typically; the cream layer was much raised. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours; very extensive separation of skin; little or no fluid.



Litmus milk tubes inoculated with grains incubated for 18 hours at 37° C., anærobically.

16. *Dried Peas (English).*—Milk 'went' typically; no butyric smell. Guinea-pig inoculated with 2 c.c. whey; died in 36 hours with separation of skin; very little fluid in the space.

17. *Dried Peas.*—Milk 'went' typically; no butyric smell. Guinea-pig inoculated with 2 c.c. whey; died in 36 hours with separation of skin.

18. *Dried Peas (Wisconsin).*—Milk 'went' typically; slight butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with extensive separation of skin; abdominal muscles sloughed through.

19. *Hemp.*—Milk 'went' typically; slight aromatic smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with extensive separation of skin; little or no fluid.

20. *Linseed.*—Milk not changed after re-incubation. There was only a firm clot. Not inoculated.

MEALS AND GRAINS TAKEN FROM FRESHLY OPENED SACKS IN A WHOLESALE WAREHOUSE

Flour in five grades of quality, No. 21 finest, 25 the lowest.

21. *Flour.*—Milk not changed.

22. *Flour.*—Milk not changed.

23. *Flour.*—Milk 'went' atypically after re-incubation. Not inoculated.

24. *Flour.*—Milk 'went'; butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; died in 16 hours; not much separation of skin.

25. *Oats (English).*—Milk 'went'; no smell. Guinea-pig inoculated with 2 c.c. whey; ulcer. Pathogenic but not fatal.

26. *Groats (whole oat grain without husk).*—Milk not changed.

27. *Oatmeal (coarse).*—Milk not changed.

28. *Oatmeal (medium).*—Milk not changed.

29. *Oatmeal (finest Irish).*—Milk 'went' violently; cream raised to cotton plug; butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours; not much separation of skin.

30. *Wheat (White English).*—Milk 'went' violently; cream raised to cotton plug; butyric acid smell. Guinea pig inoculated with 2 c.c. whey; died in 18 hours. Not much separation of skin.

31. *Wheat (Red English).*—Milk 'went' well; butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; ulcer. Pathogenic, not fatal.

32. *Flour (lowest grade).*—Milk 'went' well; butyric acid smell. Guinea-pig inoculated with 2 c.c.; ulcer. Pathogenic, not fatal.

33. *Flour (finest quality).*—Milk 'went' violently; cream raised to cotton plug; butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; died in 2 days with much separation of skin and considerable quantity of fluid in the space.

34. *Flour (Hungarian, finest quality).*—Milk tube not changed.

35. *Flour (American).*—Milk not changed.

36. *Barley (English).*—Milk 'went' well; cream raised to cotton plug; butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; died in 2 days with much separation of skin and considerable amount of fluid in the space.

37. *Darri (an Indian Grain).*—Milk 'went' well. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with considerable separation of skin.

38. *Maize.*—Milk not changed.

39. *Maize (Russian).*—Milk 'went' violently; butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with considerable separation of skin and bad-smelling fluid in the space.

40. *Beans* (*Egyptian.*)—Milk 'went' well; butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; killed; had considerable separation of skin, which had sloughed through to outside.

41. *Beans* (*English*).—Milk not changed.

42. *Rice* (*Rangoon*).—Milk 'went'; slight butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; ulcer, pathogenic.

43. *Bran.*—Supposed to be the cause of illness in some cattle; brought by farmer for examination; milk 'went' well; butyric acid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with considerable separation of tissues.

Specimens of Flower Seeds from a Seed Shop

44. *Peas.*—Milk not changed typically; putrid smell; not inoculated

45. *Peas.*—Milk not changed typically; putrid smell; not inoculated.

46. *Beans.*—Milk not changed.

47. *Timothy Grass.*—Milk not changed; clot, no gas.

48. *Cow Grass.*—Milk not changed.

49. *Rye Grass.*—Milk 'went'; putrid smell. Guinea-pig inoculated with 2 c.c. whey; died in 2 days.

50. *Rye Grass* (*Perennial*).—Milk not changed.

51. *Rib Grass.*—Milk not 'gone' typically; putrid smell. Guinea-pig inoculated with 2 c.c. whey; died in 2 days.

52. *Quaking Grass.*—Milk 'went'; putrid smell. Guinea-pig inoculated with 1 c.c. whey; ulcer. Pathogenic, not fatal.

53. *Statice* (*a fluffy grass*).—Milk 'went'; putrid smell. Guinea-pig inoculated with 2 c.c. whey; ulcer. Pathogenic, not fatal.

54. *Corn Flower.*—Milk 'went'; putrid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours, skin separated.

55. *Clover* (*White*).—Milk 'went'; putrid smell. Guinea-pig inoculated with 1 c.c. whey; ulcer. Pathogenic, not fatal.

56. *Clover* (*Red*).—Milk 'went'; putrid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with very considerable separation of skin and fluid in the space.

57. *Fennel.*—Milk 'went'; putrid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with considerable separation of skin and fluid in the space.

58. *Nasturtiums.*—Milk 'went'; much gas; putrid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with considerable separation of skin and fluid in the space.

59. *Beet.*—Milk 'went'; much gas; putrid smell. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with considerable separation of skin.

60. *Samples of dust from the top of a cupboard in the Laboratory.*—Milk inoculated 'went' typically. Guinea-pig inoculated with 2 c.c. whey; died in 18 hours with much separation of skin.

From the above 60 samples 41 gave an enteritidis-like growth in the milk; of these 30 were fatal to Guinea-pigs when inoculated, and 11 were pathogenic but not fatal.

Milk Samples (Liverpool Corporation)

Each of these samples were tested for B. enteritidis and B. coli, and all those giving an enteritidis-like growth in milk were inoculated into Guinea-pigs. All the tubes which caused a fatal result, or were pathogenic but not fatal, were accepted as

B. enteritidis. Those that were non-pathogenic were not accepted as B. enteritidis, they may have been B. enteritidis in a non-pathogenic form of BOTKIN's butyric acid bacillus.

	B. ent.? alone.	B. ent.? + B. coli.	B. coli alone.	B. ent. fatal.	B. ent. pathogenic.	B. ent. non-pathogenic.
Nov., 12 Samples ...	6	0	2	1	5	0
Dec., 50 Samples ...	18	2	0	12	5	3
Jan., 62 Samples ...	7	0	8	2	0	5
Feb., 55 Samples ...	7	1	1	0	5	3
March, 34 Samples ...	5	3	0	3	5	0

Samples sent by the Liverpool Corporation during the first four months of the year. They were all tested for B. enteritidis sporogenes and B. coli in the manner described above.

	Number of samples.	B. enteritidis alone.	B. enteritidis + coli.	B. coli alone.
Shell-fish—Cockles ...	17	7	0	0
„ Mussels ...	21	4	2	0
„ Periwinkles ...	4	4	0	0
„ Oysters ...	21	0	0	0
Tinned Meats and Fish ...	24	0	0	0
Potted Meats and Sausage ...	3	1	0	0
Butter	11	0	0	0
Margarine	3	1	0	0
Cheese	3	0	0	0
Sauces	2	0	0	0
Condensed Milk	3	0	0	0
Jams and Preserves ...	6	0	0	0
Tinned Fruits	4	1	0	0
Flour	6	3	0	0
Oatmeal	5	2	0	0

The first 20 samples of grains and the sample of dust were kindly examined by Miss CHICK for B. coli. The milk samples were examined in the usual course; the method adopted to separate the B coli was to inoculate phenol-agar plates (1-1000), which were incubated at 42° C.

If B. enteritidis had got into the samples from water or dust recently polluted with sewage matter one would have expected to find B. coli associated with it; in the case of the grains it is possible that B. coli was there and had died out, leaving the more resistant spore-bearing B. enteritidis, so the fact that no B. coli were found in the samples of grains and flour examined does not necessarily prove it never was present.

With respect to the milk and shell-fish, however, it is different; if milk is polluted with sewage matter it could only have been recently, so here one would expect to find some relation between the presence of B. enteritidis and B. coli.

In the above samples there does not appear to be any uniform relation between the two, for in the larger proportion of samples B. enteritidis occurred alone.

It is noteworthy that in the samples of grains a higher proportion of extremely virulent cultures were obtained than were got from the samples of milk and from other samples examined in the laboratory; indeed, to obtain a culture of virulent B. enteritidis it is only necessary to drop a few grains of barley or a little bran into a milk tube, and, after heating it for 20 minutes at 80° C., to incubate it at 37° C. anaerobically. The milk tube will probably be found to have undergone the typical change and to be virulent if inoculated.

Considering the frequency of its presence in the spore state on grains and seeds and its virulence when injected into Guinea-pigs, it seems certain that the B. enteritidis got there as spores and from a habitation where it preserved its virulence to a high degree.

Dust from the soil seems to be the most probable infecting agent for the grains and for most of the samples of milk; and here it may be mentioned that KLEIN found the microbe in soil and in horse manure, but not in cow manure, which may possibly be accounted for by the fact that horses are generally fed on grains, while cows seldom are.

Seeing that B. enteritidis sporogenes is even more widely distributed than has hitherto been shown and on innocent substances, two questions arise —

1. Should its presence be taken as indicating anything more than contamination by dust? and

2. Is it, after all, the causal agent of some forms of diarrhœa?

The presence of the germ in the intestinal canal may easily be accounted for both in unhealthy and healthy conditions. I am kindly allowed by Dr. GLYNN, who is working in this laboratory, to mention that he has recently found the germ in normal fæces as well as in cases of diarrhœa, and cultivations from the former were at least equally virulent.

The fact of its being virulent when injected beneath the skin of Guinea-pigs does not show that it would be pathogenic if taken by the mouth. I have fed Guinea-pigs with milk cultures, and with bran known to contain the germ capable of giving milk cultures virulent when injected, without any result.

APPARATUS FOR HEATING CULTURES TO SEPARATE SPORE-BEARING MICRO-ORGANISMS*

By C. BALFOUR STEWART, M.A., M.B. Camb., Assistant Bacteriologist to the Liverpool Corporation

Those who have occasion to work with spore-bearing micro-organisms must often have felt the want of an apparatus which would keep at a constant temperature of between 70° C. and 80° C.

An application of Meyer's hot air bath, such as is used in chemical laboratories, suggested itself as being most applicable to the purpose; several of these were made and are now kept in every-day use in this laboratory. They maintain a constant temperature of 80° C. without any attention, and are always ready for placing tube cultures in, and, what is more important, the cultures may be left without any fear of their becoming heated above 80° C.

The apparatus is similar in construction to Meyer's hot air bath, except that it is larger than those generally used and has no outlet from the bottom of the inner chamber.

The inner chamber is 18 c.m. deep and 9 c.m. in diameter, and will take 7 or 8 tubes; it is advisable to have a condensation tube of 1 m. in height.

In use.—A small quantity of pure bensole BP 80° C. is poured into the outer jacket through the hole for the condensation tube, and the tube is re-inserted. A small flame below will keep the bensole boiling, and as the vapour condenses in the condensation tube and runs back very little is lost.

The inner chamber is filled to about one-third of its depth with water at 80° C.; the water retains its heat when the lid is removed and acts as a good conductor of heat to the culture tubes when they are placed in it. If water is not put in the inner chamber the heated air escapes when the lid is removed and it takes a long

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time for the culture tubes to become heated; whereas, if water is in the inner chamber, and the culture tubes put in when the thermometer registers 80° C., it is found that there is only a fall of 10 to 15° C., and the thermometer rises to 70° C., in a few minutes.

The tubes are left in for 15 to 20 minutes after the thermometer has risen again to 70° C., so during that time they will have been kept at between 70° C. and 80° C., and for most of the time over 75° C.

The apparatus should be made in beaten copper and spun afterwards; any local coppersmith should be able to make one at the cost of about £1 5s.

DESCRIPTION OF PHOTOGRAPHS OF CULTURES OF B. PESTIS IN BROTH SHOWING 'STALACTITE' FORMATION

By Balfour Stewart, M.A., M.B. Camb.

Plate IV. Figs. 7 and 8

Fig. 7 is a photograph of a culture of B. pestis in a flask of broth; several 'stalactites' are seen growing near the side where they come into focus.

Fig. 8. 'Stalactite' growth of B. pestis in a flat flask (Soyka's flask). The light was behind, and was thrown in parallel rays by means of a lens. Between the lens and the flask was an alum bath to stop the heat rays from the incandescent lamp; without this it was found that diffusion currents were set up, and the 'stalactites' moved during the time of exposure. In front of the flask was a black screen to cut off the reflection from the edges of the flask, an opening was cut corresponding to the lower half of the flask, the upper edge of the cut out portion just allowed the surface of the broth to show.

At the left are seen several long thread-like growths ('stalactite' growth of Haffkine) hanging from small particles of butter floating on the surface.

At the right is seen a denser growth, which does not show individual 'stalactites.'

The flask was inoculated by means of a glass rod infected with plague culture. The rod was pushed down into the broth. The rod is seen to be covered with growth, which has crept up the rod and spread out over the surface of the broth, the substance of the broth remaining clear, except where some isolated colonies have grown on the sides of the flask.

EXPERIMENTS ON

THE DIFFERENTIATION AND ISOLATION FROM MIXTURES OF THE BACILLUS COLI COMMUNIS AND BACILLUS TYPHOSUS BY THE USE OF SUGARS AND THE SALTS OF BILE

By ALFRED MacCONKEY

Physiologists tell us that in the bile of animals the salts occur in proportions different to those in the human subject, and bacteriologists find that animals are not susceptible to enteric fever. It has also been noticed that intestinal fermentation is greatly lessened by the administration of 'sodium taurocholate' in keratin coated pills.

These considerations suggested a research into the possibility of using the salts of bile as a means of—

1. Differentiating the *B. c. c. from the B. t. a.
2. Isolating the B. t. a. from mixtures of organisms.
3. Finding an index of pollution of drinking waters.

For many months experiments were made to ascertain whether 'sodium taurocholate' had a different effect to 'sodium glycocholate.' The results were always the same, and it was concluded that these two salts behaved alike, until, quite accidentally, it was discovered that the chemist from whom the salts were obtained considered that the names were synonymous and had supplied mixed salts. Consequently these experiments must be considered as having been made with a mixture of the salts. The pure salts might give different results.

Elsner's medium suggested the use of potato juice as a basis in the first series of experiments, but as potato juice has not always the same composition, later an attempt was made to secure uniformity by using pure salts.

The effect of bile salts upon B. c. c. and B. t. a. may be considered under three headings:—

I. Motility.
II. Morphology.
III. Duration of life.

The medium consisted of slightly alkaline potato juice, with varying percentages of bile salts, and the incubation temperature was 37° C.

B. c. c. = Bacillus coli communis. B. t. a. = Bacillus typhi abdominalis.

Bacillus Typhi Abdominalis

I. Motility

1 p.c. Only the shorter forms are motile, and the movement is chiefly rotatory.

2 p.c. The movements are most active, and the motility is not confined to the short forms.

3 p.c. A greater number of the bacilli are actively motile.

4 p.c., 5 p.c., 6 p.c. The bacilli are actively motile in each percentage.

II. Morphology

A. Hanging drop.—The short forms preponderate. There are a few long rods and filaments. Most of the bacilli have a beaded appearance, and resemble a row of cocci. The long forms are segmented, and the segments appear to be composed of cocci. As the percentage of bile salts increases the long forms disappear.

B. Stained preparations.—The appearance of individual bacilli is much the same in each percentage. The longer forms are segmented into chains of very short bacilli, and some of the latter appear square-shaped, owing to the extreme ends not taking up the stain.

Bacillus Coli Communis

I. Motility

1 p.c. Actively motile.

2 p.c. Motility about the same.

3 p.c. Slight decrease in motility.

4 p.c., 5 p.c., 6 p.c. Motility decreases with the increased percentage of bile salts until it is doubtful whether any of the bacilli are motile. They shew a tendency to 'clump.'

II. Morphology

A. Hanging drop.—1 p.c. The bacilli are not to be distinguished from the B. t. a. except in that the short forms have a more oval appearance.

2 p.c. to 6 p.c. With the increase in amount of bile salts the length of the bacilli decreases until they appear to be cocci and very short rods.

B. Stained preparations.—The staining is very irregular. Much more so than in the case of B. t. a. Frequently the ends of a bacillus do not stain and are somewhat swelled, while in the centre is a very short stained portion which often looks like a coccus.

A bile salt potato juice gelatine was used for plate, stab, and slant cultures, but there was nothing characteristic, either in the mode of growth or in stained preparations, except in the case of one strain of B. c. c., which always, after 24 hours' growth, showed many huge fantastic-shaped involution forms which stained deeply. Many other strains were grown, but the same result was never obtained. Lactose, glucose, and sucrose were added to the gelatine and used in slant cultures, but no characteristic differences were visible.

Duration of Life

As bile salts had an inhibiting effect upon the motility of an actively motile B. c. c., while that of B. t. a. was unaffected, it was thought that possibly B. t. a. might live longer than B. c. c. in the bile salt medium.* In the first experiment the two strains of B. c. c. used died in 9 days, while the B. t. a. lived for some time longer. In this and subsequent experiments the incubation temperature was 42° C., so that if a successful result was obtained the temperature would assist in inhibiting the growth of other organisms. The various media used and the duration of life of B. c. c. and B. t. a. are given in the appended Table:—

Organism and Source	Bile salt 4% Pot. Juice	Bile salt 4% Pot. Juice	Bile salt 4% Glucose 2.5% Pot. Juice	Bile salt 5% Glucose 2.5% Pot. Juice	Bile salt 5% Glucose 2.5% Pot. Juice	Bile salt 5% $NaNO_3$ 1.5% Water	Bile salt 5% KNO_3 1.5% Water
B. t. a. (Laboratory)	L 16	L 12	D 4	D 5	D 5	D 4	D 5
„ Human fæces	L 16	L 12	D 2	D 5	D 5	D 4	D 5
B. c. c. isolated by Eyre from H_2O...	D 6	L 12	D 7	D 8	D 6	D 7	D 6
„ given by Pakes	D 6	D 4	D 4	D 8	D 6	D 5	D 11
„ isolated from H_2O by Pakes	L 16	L 12	L 8	D 8	D 4	D 7	D 5
„ „ „ human fæces...	L 16	D 8	L 8	D 8	D 4	D 7	contaminated
„ „ „ „ „ ...	L 16	L 12	D 4	D 8	D 7	D 8	D 10
„ „ „ horse „ ...	L 16	D 8	L 8	D 8	D 4	D 7	D 5
„ „ „ cow „ ...	L 16	L 12	D 8	D 8	D 4	D 7	D 5
„ „ „ sheep „ ...	D 6	D 6	L 8	D 8	D 5	D 7	D 5
„ „ „ rabbit „ ...	L 16	L 12	D 7	D 8	D 6	L 11	D 5
„ „ „ dog „ ...	—	—	D 6	—	D 6	—	D 5
„ „ „ „ „ ...	—	—	D 6	—	D 4	—	D 5
„ „ „ mouse „ ...	—	L 12	D 4	D 8	D 8	L 11	D 13
„ „ „ Ice Cream by Pakes ...	D 6	L 12	L 8	D 8	D 11	D 5	D 5
„ „ „ blood of case of Infective Endocarditis ...	—	—	D 7	—	D 4	—	D 7
Typhoid stools	—	—	D 5	—	—	—	L 15
Gærtner, given by Durham... ...	L 16	L 12	L 8	D 8	D 12	L 11	L 15

L = living. D = dead. The numbers indicate the days after inoculation.

In order to ascertain whether a culture was living the bile salt tube was well shaken, and a loopful transferred to ordinary broth, and this broth tube incubated at

* A percentage of 4 was used because the effect upon the motility of B. c. c. was distinct.

37° C. If the broth cultures did not show any growth the transplantations from the bile salt medium were still continued for several days, so as to make quite certain that there were no viable organisms present.

The results are negative as regards both differentiating B. t. a. from B. c. c., and isolating B. t. a. from mixtures. But they show that 2.5 p.c. of glucose has a distinctly inhibiting effect upon the organisms used.

This action of glucose is confirmed by the following experiment. Fæces from

1. A case of Bright's Disease,
2. A case of Mitral Stenosis,
3. A case of Cirrhosis with ascites,

were inoculated into tubes containing

A. Bile salt 5 p.c. KNO_3 1.5 p.c. Water,
B. Bile salt 5 p.c. $NaNO_3$ 1.5 p.c. Water,
C. Bile salt 5 p.c. Glucose 2.5 p.c. Potato Juice,

and incubated at 42° C.

1 *A.* Was living on 14th day.
1 *B.* Was living on 14th day.
1 *C.* Was dead on 5th day.
2 *A.* Was living on 14th day.
2 *B.* Was living on 14th day.
2 *C.* Was dead on 5th day.
3 *A.* Was living on 14th day.
3 *B.* Was living on 14th day.
3 *C.* Was dead on 6th day.

The latest culture which shewed any growth was plated in ordinary nutrient gelatine.

The organisms isolated were—From

1 *A.* An organism which looked like a streptococcus, and which gave
Broth—General turbidity,
Litmus Milk—Acid, but no clotting,
Potato—Raised whitish growth,
Gel. S. tab—Very slight growth on surface, but good growth all down the needle track.

1 *B.* B. c. c.

1 *C.* (*a*) B. c. c.
(*b*) A bacillus which produced slight fluorescence in gelatine, caused no change in milk, and gave a moist yellowish growth, with discolouration of potato.

2 *A.* Organism similar to 1 *A.*

2 *B.* B. c. c.

2 *C.* (*a*) B. c. c.
(*b*) Organism similar to 1 *C.*

3 *A.* Liquefying motile bacillus, which peptonizes milk, turning it brownish yellow, gives slight indol, but no gas; produces general turbidity in broth, and a moist brownish-yellow growth, with discolouration of potato.

3 *B.* Same as 3 *A.*

3 *C.* B. c. c.

From these three cases, then, there were isolated four organisms which would grow in bile salt 5 p.c. media at 42° C., and the most frequent was the B. c. c.

It was thought that an increased percentage of bile salt might inhibit all but B. c. c. [That B. c. c. would grow in 7 p.c. B. s. was proved by the experiment given in the following Table :—

Medium—Bile salt 7 p.c., KNO_3, $NaNO_3$, AmCl., each 0.5 p.c. Made up with water. Temperature—42° C.

B. t. a. fæces . . .	Dead 5th day.
B. c. c.	Living 10th day.
„ Infec. Endocarditis . .	„ „
„ Ice Cream . . .	„ „
„ Cow . . .	„ „
„ Horse . . .	„ „
„ Sheep . . .	„ „
„ Dog . . .	„ „
Gærtner	„ „]

So fæces from (1) a case of phosphorous poisoning, (2) a case of (?) influenza, were inoculated into tubes containing the above medium, and incubated at 42° C. for 5 days, and then plates were made from these tubes.

From case 1 were obtained (*a*) a liquefying motile bacillus, similar to the one which had been isolated from previous case ; (*b*) a non-typical B. c. c. From case 2, only B. c. c. was isolated.

Fæces from the Rabbit, Sheep, and Cow were inoculated into the same medium, and incubated at 42° C., for 48 hours, and then plated in ordinary gelatine.

The organisms isolated were :—From Rabbit, only B. c. c. group ; Sheep, B. c. c., and the same liquefying motile bacillus as before ; Cow, B. c. c. group only.

The experiment was repeated with two specimens of Rabbit fæces, and the organisms isolated were :—(*a*) B. c. c. ; (*b*) a motile liquefying bacillus having the same characteristics as before.

A repetition with fæces of the Cow and Sheep resulted in the isolation of B. c. c. only.

As glucose seemed to have an unfavourable effect upon the duration of life of B. c. c. and B. t. a. a liquid medium was made, consisting of potato juice containing varying percentages of glucose, and B. c. c. and B. t. a. were grown in this medium at 37° C. The amounts of the sugar used were 1, 2, 3, and 4 per cent.

Bacillus Typhi Abdominalis

Motility

The lowest percentage causes a decrease in the motility as compared with ordinary broth, and as the percentage increases the amount of movement decreases.

Morphology

In 1 p.c. the bacilli have much the same appearance as in ordinary broth, but the size decreases with the increase in the amount of glucose.

Bacillus Coli Communis

Motility

A fairly motile B. c. c. was used, and it was found that motility decreased with an increased percentage, until in 4 p.c. it was doubtful whether the bacilli were motile.

Morphology

The bacilli become shorter as the percentage rises.

B. Enteritidis of Gærtner

The effect of glucose upon this organism is the same as upon B. t. a. and B. c. c.

[All the experiments up to this point were carried out in the Bacteriological Laboratory at Guy's Hospital during the winter of 1897 and the first half of 1898. Then an interruption occurred and they were not resumed until 1900, in the Thompson Yates Laboratories, University College, Liverpool.]

The conclusion arrived at from the first series of experiments was, that by using a liquid bile salt medium, an incubation temperature of 42° C., and subsequently plating in ordinary nutrient gelatine, one could inhibit the growth of most of the organisms usually found in fæces. The thought naturally arose that possibly some of the organisms isolated were merely existing in the bile salt medium without multiplication, and that it was the subsequent plating in a more favourable medium that enabled them to grow. Therefore an agar medium was tried; at first without peptone, but later, when it was seen that peptone did not increase the number of species, a slightly alkaline agar, composed as follows, was used :—

Bile salt	1 gramme.
Peptone	0.2 „
KNO_3	0.5 „
Na_2HPO_4	0.5 „
AmCl	0.5 „
Agar	2 „
Tap water	100 c.c.

When used for sewage effluents this medium gave an almost pure culture of the B. c. c. group; but when used for pure cultures of B. c. c., and compared with nutrient agar, the bile salt medium frequently inhibited too much.

The following are examples of the results obtained :—

I. About two grammes of garden earth were shaken up with about 100 c.c. of tap water in a flask, which was then allowed to remain at rest until the larger particles

had settled to the bottom. The supernatant liquid was plated, 1 c.c. being put into each plate. The plates were incubated at 42° C., and examined after 48 hours.

Nutrient Agar	.	The plate was covered with colonies of various kinds.
Bile Salt Agar	.	There was no growth in this plate.

II. Pure cultures of B. c. c. (*A*, *B*, *C*, and *D*) were diluted with sterile water, and 1 c.c. of the dilution was put into each plate.

A. *Nutrient Agar*			
3 months old.	1 . .	135 colonies	Average 138
	2 . .	104 „	
	3 . .	175 „	
Bile Salt Agar			
	1 . .	65 colonies	Average 103
	2 . .	151 „	
	3 . .	94 „	
B. *Nutrient Agar*			
1 month old.	1 . .	356 colonies	Average 562
	2 . .	912 „	
	3 . .	420 „	
Bile Salt Agar			
	1 . .	570 colonies	Average 536
	2 . .	500 „	
C. *Nutrient Agar*			
12 days old.	1 . .	35 colonies	Average 27
	2 . .	29 „	
	3 . .	19 „	
Bile Salt Agar			
	1 . .	16 colonies	Average 23
	2 . .	28 „	
	3 . .	25 „	
D. *Nutrient Agar*			
24 hours old.	1 . .	116 colonies	Average 118
	2 . .	136 „	
	3 . .	102 „	
Bile Salt Agar			
	1 . .	57 colonies	Average 77
	2 . .	121 „	
	3 . .	53 „	

When endeavouring to estimate the value of this medium, in the case of mixtures containing B. c. c., a difficulty arose from the fact that nutrient agar cannot be used as a standard. So for sewage, river water, &c., carbolic agar, 1 °/$_{oo}$ was taken as the standard, because it is said that carbolic acid in this percentage practically gives the same results as nutrient agar in the case of pure cultures of B. c. c.

Several samples (about 12) of Severn water, from the neighbourhood of Shrewsbury, were plated in this bile salt agar, and in carbolic agar 1 °/∞. The results showed that the bile salt agar inhibited some 70 p.c. of the B. c. c. The problem, then, was to find out the composition which would give results equal to carbolic agar.

Numerous alterations in, and additions to, the medium were made, and in the course of these experiments it was found that—

Bile Salts—1 p.c. and over—have an inhibitory effect upon the great majority of organisms, but favour the growth of moulds.

KNO_3, AmCl, NaCl—0.5 p.c.—exercise an unfavourable influence upon B. c. c. while encouraging the growth of other organisms.

Na_2HPO_4—0.5 p.c.—seems specially favourable to earth organisms.

Glucose—0.5 p.c.—tends to inhibit the ordinary earth and water bacteria, and, if in a larger percentage, B. c. c. also; but assists the growth of moulds and yeasts. A small percentage of glucose benefits B. c. c., especially the colonies in the depth.

One example will show the effect of glucose and of salts upon B. c. c. A pure culture of B. c. c. was taken and diluted with sterile water, plated, and incubated at 42° C.

	Nutrient Agar	Bile salt 1% Peptone 0.2%	Bile salt 1% Peptone 0.2% AmCl. 0.5% KNO_3 0.5%	Bile salt 1% Peptone 0.2% Glucose 0.5%	Bile salt 1% Peptone 0.2% Glucose 1%	Bile salt 1% Peptone 0.2% Glucose 1.5%
	870	440	260	960	496	270
	700	230	200	800	600	240
	960	300	168	650	484	228
Average No. of Colonies per plate	844	323	209	803	527	246

If B. c. c. be plated in a medium containing bile salts, peptone, and glucose only, the colonies at the end of 24 hours appear much larger than usual, and have a hazy outline, owing to the formation of a precipitate round each colony. If there are many colonies the plate looks 'milky,' and has a yellowish colour. This precipitate is caused by an acid produced by the organism. If a drop of NH_3 solution be placed upon the colony the haze soon disappears, and the colony is seen to be of the usual size. The addition of salts to the medium tends to prevent the formation of this precipitate, and thus to remove a means of distinguishing one kind of colony from

another. Further experiments showed that a percentage of glucose not greater than 0.5 was most favourable, and that an increase of the peptone to 1 p.c. did not cause an increase in the number of organisms other than B. c. c. So a medium composed of bile salts 1 p.c., peptone 1 p.c., glucose 0.1 p.c., agar 2 p.c., and tap water, was compared with carbolic agar 1 p.c. ; 16 samples of Severn water being plated. The results were, however, disappointing as there was no definite correspondence between the numbers in the two media. In a few of the samples the numbers were practically the same, but in the others the bile salt agar results were much too low.

An increase in the amount of bile salt caused a decrease in numbers, and the addition of 0.5 p.c. of carbolic acid decidedly inhibited. Thus a pure culture of B. c. c. gave—

	Nutrient Agar	Bile salt 1% Peptone 1% Glucose 0.3% Carb. Acid 0.5%	Bile salt 1% Peptone 1% Glucose 0.3%	Bile salt 1.5% Peptone 1% Glucose 0.3%	Bile salt 2% Peptone 1% Glucose 0.3%
	109	20	67	30	33
	102	15	101	23	36
	78	12	47	21	31
Average No. of Colonies per plate	96	15	71	24	33

On some occasions the addition of KNO_3 0.1 p.c. seemed to increase the numbers of B. c. c., and on other occasions the numbers were decreased. So a series of trials was made with percentages of KNO_3, varying from 0.1 to 0.5, and the conclusion come to was that this salt had an unfavourable effect upon the growth of B. c. c. Just prior to coming to this conclusion 20 samples of Severn water were plated in bile salt 0.5 p.c., peptone 2 p.c., glucose 0.5 p.c., KNO_3 0.1 p.c., agar 1.5–2 p.c., tap water q. s. The results corresponded more closely than before with those given by carbol agar ; but the inhibition was still too great.

At this stage Dr. HERBERT E. DURHAM suggested that I should once more try the effect of different kinds of sugars. The hypothesis he enunciated (as far as I understood it) was that if an organism ferments a sugar it presumably gives rise to products which are inimical to its own growth, and that, consequently, if of two organisms *A* and *B*, *A* ferments both glucose and lactose, while *B* only ferments glucose, then a medium containing lactose should be more favourable to *B* than to *A*. Glucose, lactose, and mannite were the sugars tried, and it seemed that when employed

in the same percentage—0.5—glucose was most favourable to B. c. c.; but that 1 p.c. of lactose gave results equal to 0.5 p.c. glucose. It was doubtful whether mannite had an equal inhibitory effect upon other organisms.

As nitrates, chlorides, and phosphates seemed unfavourable, it was thought that possibly Liebig's Extract might be of use in giving the extra amount of nourishment apparently required. So garden earth and road sweepings were thoroughly shaken up with tap water, and the mixture plated in

I. B. s. 0.5 p.c., peptone 2 p.c., lactose 0.5 p.c., agar 1.5 p.c.

II. B. s. 0.5 p.c., peptone 2 p.c., lactose 0·5 p.c., agar 1.5 p.c., Liebig 0.1 p.c. Nutrient agar being used as a control.

The control plate was covered with all kinds of colonies.

I. The plates appear to contain only one kind of organism. On one of them there were 12 surface colonies. Eleven of these were subcultured in broth, and afterwards in milk, glucose jelly, and potato.

	Broth	Milk	Glucose Jelly	Potato	Indol
1.	General turbidity	Acid and clotting	Profuse gas No liquefaction	Moist, grey growth. P.D.	
2. Actively motile. Short bac.	,,	,,	,,	Yellowish P.D.	+
3. ,, ,, ,,	,,	,,	,,	,, ,,	+
4. Motile ,,	,,	,,	,,	,, ,,	+
5. Actively motile ,,	,,	,,	,,	,, ,,	+
6. ,, ,, ,,	,,	,,	,,	,, ,,	+
7. Motile ,,	,,	,,	,,	,, ,,	+
8. Actively motile ,,	,,	,,	,,	Almost invisible	+
9. ,, ,, ,,	,,	,,	,,	Moist, grey. P.D.	+
10. ,, ,, ,,	,,	,,	,,	Yellowish ,,	+
11. ,, ,, ,,	,,	,,	,,	,, ,,	+

P.D. = discolouration of potato.

II. The plates appear to be a pure culture. The numbers are practically the same as in I. There were 11 surface colonies on one plate. These were all subcultured in broth, and later in milk, glucose jelly, and potato.

YEAGLI JHAI

	Broth	Milk	Glucose Jelly	Potato	Indol
1. Motile. Short bac. .	General turbidity	Acid and clotting	Gas No liquefaction	Moist, grey yellow. P.D.	+
2. „ „	„	„	„	„ „	+
3. Slightly motile. Short bac.	„	„	„	Almost invisible	+
4. Actively „ „	„	„	„	Moist, yellow. P.D.	+
5. „ „ „	„	„	„	„ „	+
6. ? Motile „	„	„	„	„ „	+
7. Actively motile „	„	„	„	„ „	+
8. Slightly „ „	„	„	„	Moist, grey yellow. P.D.	+
9. Motile „	„	„	„	Moist, grey. P.D.	+
10. Actively motile „	„	„	„	Almost invisible	+
11. Motile „	„	„	„	Moist, yellowish. P.D.	+

P.D. = discolouration of potato.

Again, 5 c.c. of the Ashton Hall Septic Tank liquor were mixed with 95 c.c. of the earth, road sweepings, and tap water mixture used in the previous experiment, and which had been standing on the laboratory bench. 1 c.c. of the mixture was put into each plate, and the medium used was B. s. 0.5 p.c., peptone 2 p.c., lactose 1 p.c., agar 1.5 p.c., tap water. The average of three plates was 1194 colonies per plate. The whole of the plate was ' milky,' except for a small clear space round some of the colonies. The colonies with a clear space round them appeared to be of two kinds. One was finely and uniformly granular, with a regular, well-defined margin ; the other had an irregular edge, and was covered with fine wavy lines. Cultures in broth were made from eight colonies.

		Broth	Milk	Glucose Jelly	Potato	Indol
1. Granular colony with clear space.	Short B., motile	General turbidity	Acid and clotting	Gas No liquefaction	Moist, grey P.D.	+
2. „ „	„ ? motile	„	„	„	„	+
3. „ „	„ ? motile	„	„	„	„	+
4. „ „	„ motile	„	„	„	„	+
5. Hazy colony. .	„ „	„	„	„	„	+
6. „ „	„ actively motile	„	„	„	„	+
7. Lined „	„ „	„	„	„	„	+
8. „ „	„ motile	„	„	„	„	+

The flask containing the mixture of earth, road sweepings, and tap water was allowed to stand on the laboratory bench for several days. Then 100 c.c. of the supernatant liquid were poured into another flask, and shaken up with about 2 grammes of fresh garden mould. This mixture was inoculated with 1 loopful of a culture of B. t. a., and the flask, unplugged, placed in a dark cupboard for two days at ordinary temperature. Plates were then made, about 0.25 c.c. being put into each plate, in

I. B. s. 0.5 p.c., peptone 2 p.c., glucose 0.5 p.c., agar 1.5 p.c.

II. B. s. 0.5 p.c., peptone 2 p.c., lactose, 0.5 p.c., agar 1.5 p.c.

III. B. s. 0.5 p.c., peptone 2 p.c., lactose 1 p.c., agar 1.5 p.c.

I. 45 colonies on the plate. On the surface were 9, which were all transferred to broth, and subcultures made.

	Broth	Milk	Glucose Jelly	Potato	Indol
1. Short bac. Actively motile	General turbidity	Acid, clotting	Gas No liquefaction	Moist, yellowish P.D.	?
2. " " "	"	No change	No gas No liquefaction	'Invisible'	None
3. " " "	"	"	"	"	"
4. " " "	"	"	"	"	"
5. " " "	"	"	"	"	"
6. " " "	"	"	"	"	"
7. " " "	"	"	"	"	"
8. " " "	"	Acid, clotting	Gas No liquefaction	Moist, grey. P.D.	?
9. " " "	"	No change	No gas No liquefaction	'Invisible'	None

II. 51 colonies, of which 7 were on the surface.

	Broth	Milk	Glucose Jelly	Potato	Indol
1. Short bac. Actively motile	General turbidity	No change	No liquefaction No gas	'Invisible'	?
2. " " "	"	"	"	"	None
3. " " "	"	Acid, clotting	No liquefaction Gas	Grey yellow, moist. P.D.	"
4. " " "	"	No change	No liquefaction No gas	'Invisible'	"
5. " " "	"	"	"	"	"
6. " " "	"	"	"	"	"
7. " " "	"	"	"	"	?

This plate was exposed to light for several days, when one of the surface colonies became yellow.

III. There were 122 colonies on this plate. It was exposed to light for several days. Three of the surface colonies turned yellow; the rest remained greyish and translucent. The 3 yellow colonies and 3 others were transferred to broth.

Colony	Organism	Broth	Milk	Glucose Jelly	Potato	Indol
1. Yellow . .	Short bac., actively motile	General turbidity	Clotting and acid	No liquefaction Gas	Moist, yellow. P.D.	+
2. „	„ „	„	„	„	„	+
3. „	„ „	„	„	„	„	+
4. Grey . . .	„ motile	„	Slight acid No clotting	No liquefaction No gas	Just visible	+
5. „	„ „	„	„	„	„	+
6. „	„ „	„	„	„	„	+

The mixture of earth, &c., remained in the cupboard for five more days, when it was again plated on nutrient agar and on lactose bile salt agar. On the nutrient agar plate there were about 70 colonies of various kinds, of which five or six might have been B. c. c. or B. t. a.

On the bile salt agar plate there were four colonies. Cultures were made from two of them. Both gave cultural characteristics corresponding to the B. t. a. One was tested with serum and gave a positive reaction; it also gave the indol reaction.

Five days later this mixture was again plated in bile salt lactose agar, 1 c.c. being put into the plate. Only one colony, besides a few moulds, appeared, and this was B. c. c.

One c.c. of urine, from a case* of enteric fever, was put into a plate of bile salt lactose agar. The result was an almost pure culture of B. c. c. A few of the colonies seemed as if they might be B. t. a., and these were transferred to broth and subcultured.

	Broth	Milk	Glucose Jelly	Agar	Indol
1. Slender bac. Extremely motile	General turbidity Greenish yellow fluorescence	Acid clotting Peptonization Greenish yellow liquid	No Gas liquefaction Greenish yellow colour	Bac. Pyocyaneus	+
2. „ „ „	„	„	„	„ „	+
3. „ „ „	„	„	„	„ „	+
4. Short bac. Slightly motile .	General turbidity	Acid and clotting	No liquefaction Gas	Dry, filmy, greyish translucent growth } No spores. Not by Gram.	+
5. „ „ „	„	„	„	„ „	+

* This case was a typical case subsiding by lysis.

The specimen was taken on the 24th day. The temperature was normal. The serum reaction had been tried 3 times, with 2 negative results and 1 doubtful.

Urine from another enteric case, which was convalescing from a relapse, was taken, and 1 c.c. put into a plate of bile salt lactose agar. Five cultures were made from this plate.

		Broth	Milk	Glucose Jelly	Potato	Indol
1.	Short bac. Actively motile.	General turbidity	Acid	Gas No liquefaction	Just visible	+
2.	„ ? motile . .	„		„	Moist, yellow growth with discolouration	+
3.	„ „ . .	„	Acid, clotting	„	„	+
4.	„ „ . .	„		„	„	+
5.	„ „ . .	„		„	„	+

None of these formed spores; nor did they stain by Gram's method.

Again, fæces from a case of enteric, which was in the 3rd week of a relapse and 10th week of the disease, were diluted 30,000 times with sterile water and plated. 1 c.c. of this dilution was put into each plate. The plates were overcrowded with what looked like a pure culture of the B. c. c. group. Subcultures were made from 19 surface colonies.

	Motility	Broth	Glucose jelly	Milk	Potato	Indol
1. Short bacillus	?	General turbidity	Gas No liquefaction	Acid clotting	Moist, transparent	+
2. „	+	„	„	„	Moist, yellow. P.D.	+
3. „	?	„	„	„	„ „ „	+
4. „	+	„	„	„	„ grey, yellow. P.D.	+
5. „	?	„	„	„	„ transparent	+
6. „	−	„	„	„	„ „	+
7. „	−	„	„	„	„ grey, yellow. P.D.	+
8. „	−	„	„	„	„ „ „ „	+
9. „	+	„	„	„	„ „ „ „	+
10. „	+	„	„	„	„ „ „ „	+
11. „	+	„	„	„	„ „ „ „	+
12. „	?	„	„	„	„ „ „ „	+
13. „	?	„	„	„	'Invisible'	+
14. „	?	„	„	„	Moist, grey, yellow. P.D.	+
15. „	?	„	„	„	„ „ „ „	+
16. „	?	„	„	„	„ transparent	+
17. „	+	„	„	„	„ „	+
18. „	+	„	„	„	„ grey, yellow. P.D.	+
19. „	+	„	„	„	„ „ „ „	+

P.D. = discolouration of potato.

The following samples were plated in Bile salt 0.5 p.c., peptone 2 p.c., lactose 1 p.c., agar 1.5 p.c. :—

I. Road puddle diluted with sterile water. Carbol agar (Miss CHICK's analysis).

1. 200 B. c. c.
2. 212 „
3. 174 „

= 195 . . Average 211

II. Road dust, shaken up with sterile water.

1. 13 B. c. c.
2. 11 „
3. 9 „

= 11 . . Average 3

III. Earth from a potato field, shaken up with sterile water.

1.
2.
3.

0 B. c. c. . . . . Average 0

IV. Earth from another potato field, shaken up with sterile water.

1.
2.
3.

Sterile, except for a few moulds . Average 0

V. Garden earth, in which turnips were growing, shaken up with sterile water.

1. 1 B. c. c.
2. 1 „
3. 1 „

= 1 . . . Average 3

Samples I, II, and III contained, besides B. c. c., another kind of colony which was very small, round, grey, slightly raised, and looked finely granular under the microscope. Eleven cultivations were made from these colonies, and all turned out to be yeasts.

Plates were also made with water from the aqueducts at Vyrnwy and Rivington. Three plates were made from each sample, 1 c.c. of water being put into each plate.

There was no growth on any of these plates.

A sample of water from a pond, in which there were 20,000 young trout, was plated in the same medium, 1 c.c. being put into each of 3 plates. There was no growth on any of the plates.

Countings were made of 13 samples of Severn water, and it was found that when there were not many colonies on a plate the number agreed with those on carbol agar ; but when the colonies were more than about 100 per plate other organisms interfered with the proper development of the B. c. c.

These results are a fair reason for concluding that by means of a lactose bile salt agar, and an incubation temperature of 42° C., one can inhibit almost all the intestinal organisms except B. c. c., and that B. c. c. will grow as well on this medium as on carbol agar.

But the correctness of this conclusion is rendered doubtful by the following experiments :—

A pure culture of the Escherich strain of B. c. c. (given me by Dr. HERBERT DURHAM) was plated in nutrient agar, carbol agar, and lactose bile salt agar.

In the first experiment the solution was not dilute enough, and the nutrient agar and carbol agar plates were overcrowded, there being more than 1,600 colonies on a plate. But even with this large number present in the solution not more than 20 had grown on a lactose agar plate.

In a second experiment the numbers were :—

Nutrient agar	Carbol agar 1 °/∞	Bile salt lactose agar
324	52	5 plates
338	51	No growth
332	40	
331	48	

In a third similar experiment the numbers were :—

Nutrient agar	Carbol agar 1 °/∞	Bile salt lactose agar
161	9	3 plates
172	9	No growth
89	0	
140	9	

Similar experiments made with a pure culture of B. t. a. gave results :—

I

Nutrient agar	Carbol agar 1 °/∞	Bile salt lactose agar
Overcrowded, more than 3,000 per plate.	2 per plate.	Overcrowded, more than 3,000 per plate.

II

Nutrient agar	Carbol agar 1 °/∞	Bile salt lactose agar
139	0	129
166	0	143
133	0	142
146	0	138

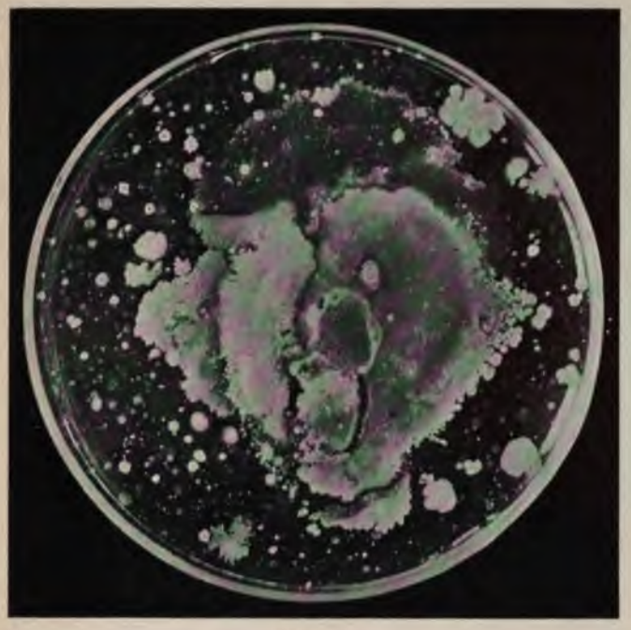


FIG. 1

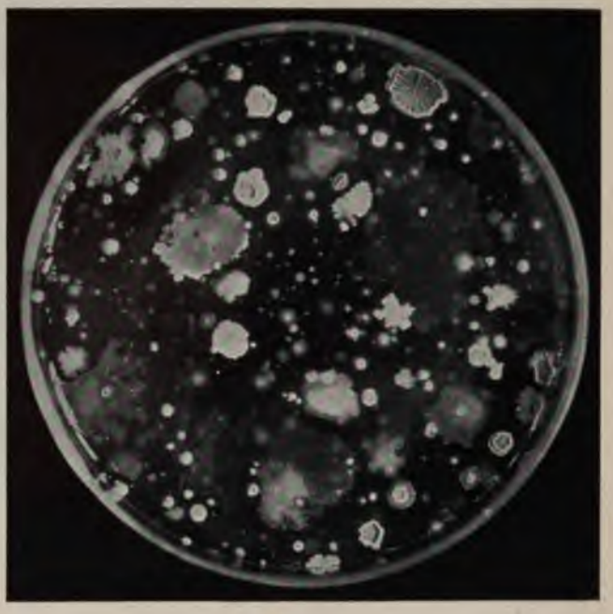


FIG. 2

FIG. 3

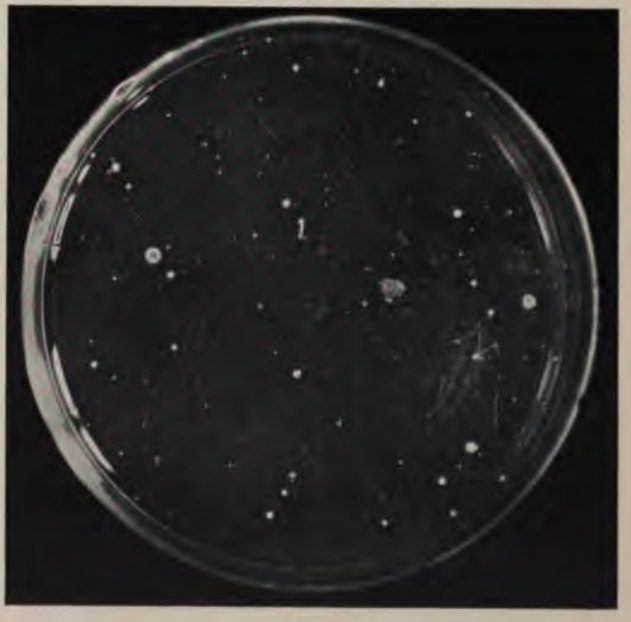
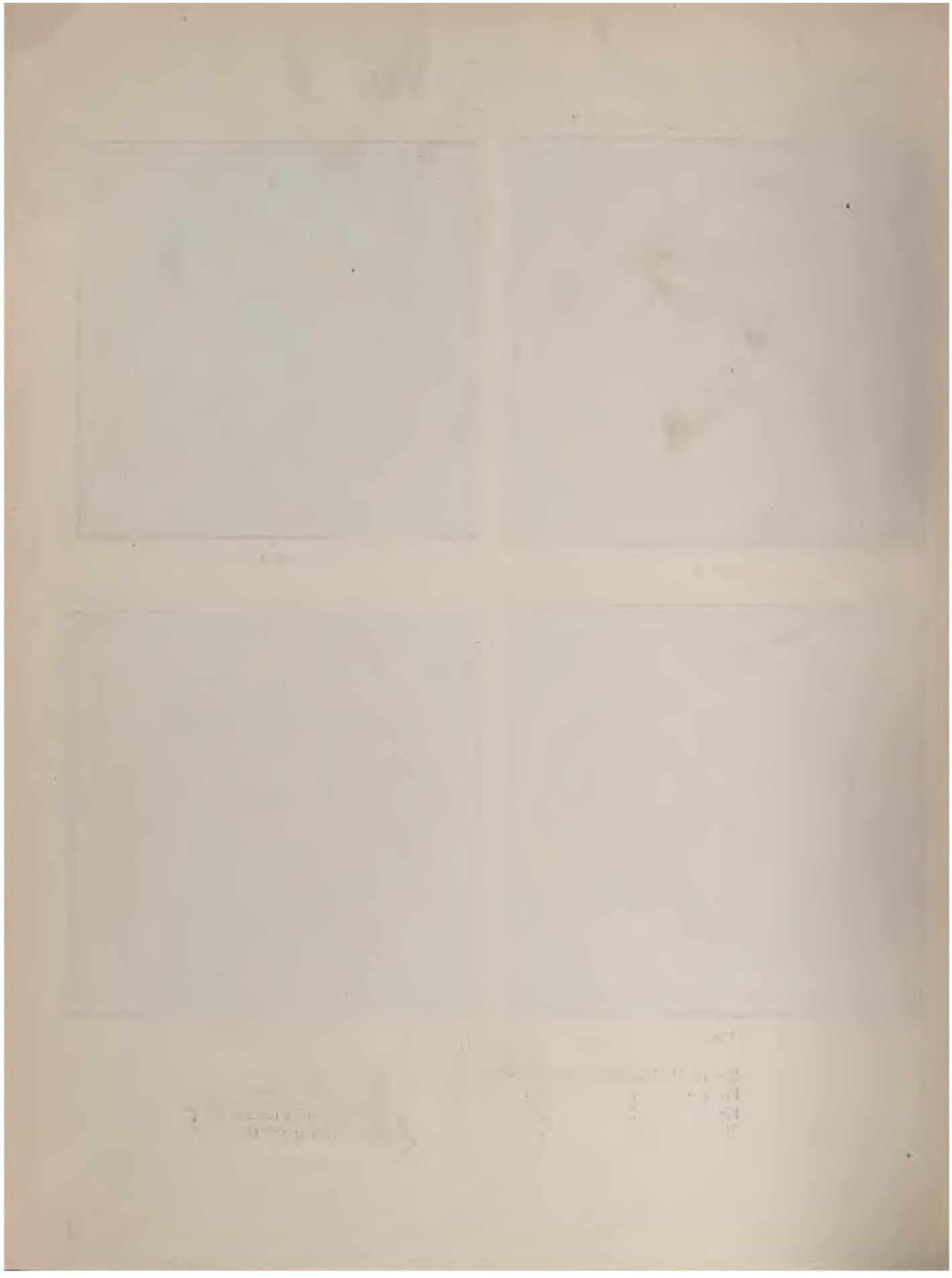


FIG. 4

Fig. 1—Garden mould + tap water + B. coli com. Nutrient agar at 42° C.
Fig. 2 " " " Nutrient agar at 37° C.
Fig. 3 " " " Carbol agar 1 in 1000 at 37° C.
Fig. 4 " " " Bile-salt agar at 37° C.



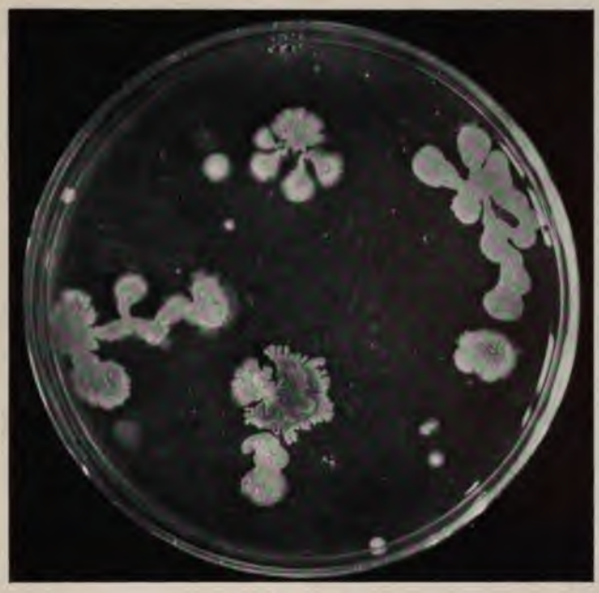


FIG. 5

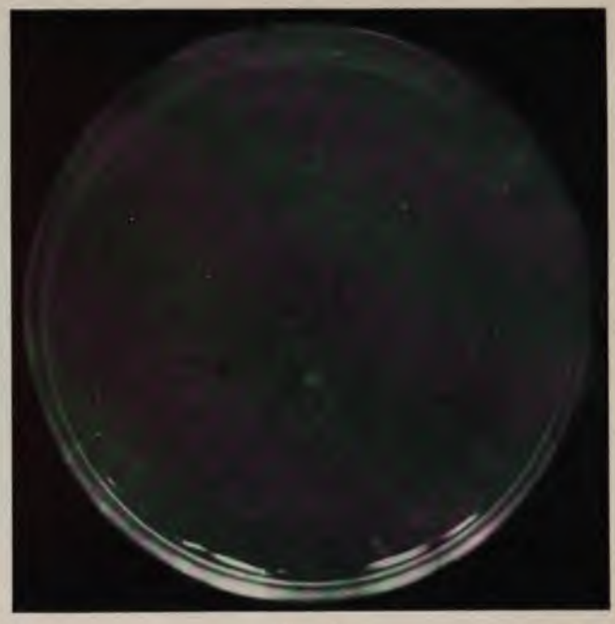


FIG. 6

FIG. 7

FIG. 8

Fig. 5—Sewage contaminated mud. Carbol agar 1 in 1000.
Fig. 6—Sewage contaminated mud. Bile-salt agar.
Fig. 7—Stalactite growth of B. pestis, natural size.
Fig. 8—Stalactite growth of B. pestis.

These experiments are so few that a definite conclusion cannot be framed, but they suggest that working on these lines it may be ultimately possible to devise a simple method of isolating the Bacillus typhi abdominalis.

In making up the medium great care must be taken not to overheat it after adding the lactose, as, in that case, a change takes place, and B. t. a. produces a haze like B. c. c. The composition which has given the best results so far is :—Bile salt 0.5 p.c., peptone 2.0 p.c., lactose 1.0 p.c., agar 1.5 p.c., tap-water q. s.

NOTE UPON THE

ACTION OF THE DIBDIN CONTACT BEDS CONSTRUCTED BY THE CORPORATION OF LIVERPOOL AT WEST DERBY

The following account of the Sewage Farms at West Derby and Walton, and of the subsequent construction of Storm Water Filter Beds, and of Dibdin Beds, has been kindly furnished by the City Engineer. I desire to express my thanks both to the City Engineer, the Assistant Engineer (Mr. COOPER), and the Farm Bailiff (Mr. SMITH), and more recently to Mr. EDGE, for their cordial co-operation in enabling me to conduct the bacteriological investigations.

The interest attaching to the experiments lies in the comparison of the sand effluents with the Dibdin effluents, and with the action of the rough storm water filter beds, which have been in use since April, 1896. The bacteriological analyses have been made by Drs. HILL, MACCONKEY, and Miss CHICK.

RUBERT BOYCE.

COPORATION SEWAGE FARMS AT WEST DERBY & WALTON

The system adopted on these Farms is broad irrigation, and filtration without chemical treatment. On the West Derby Farm special contact beds have been constructed.

The West Derby Farm has an area of 207 acres, which, at the present time, takes the sewage of about 35,000 inhabitants, spread over an area of about 2,000 acres. The sewage is conducted on to the Farm by means of two sewers, one a high-level brick sewer, 5ft. 3in. by 3ft. 6in. in size, outlets at the highest point of the land, whence, if desired, the sewage could be carried to any part of the Farm. It is a gravitation sewer throughout, with a ruling gradient of 5 feet per mile, or 1 in 1,056. The second sewer, which is also of brick, but 4 ft. 6 in. by 3 ft. internal dimensions, with a gradient of 1 in 1,120, serves the low-lying portion of the district, and discharges into a tank some 13 feet below surface level at the lowest part of the land. It has here to be raised by pumping to a height of about 10 feet above the ground level, to permit of its distribution over the land by gravitation. This distribution is effected both for the high and low level sewers by means of open carriers (or conduits),

which are generally constructed of concrete, but where the transverse depressions in the land are, of galvanized iron, supported on tressels. Syphons are provided under any cartways which are encountered in the course of the carriers. Special provision is made for storm water, which mostly enters the sewers (as few surface water drains exist), and in storm time reaches the Farm more rapidly than it can be filtered through the land.

The provision for storm water consists of a plot of land some 20 acres in extent, which is banked round to the height of 3 feet. The storm water accumulates on this plot, and is gradually run off through a Coke Screening Chamber with double screens, filled with coke of small size, which suffices to arrest most of the suspended matter in the diluted sewage; and also filter beds composed of burned clay ballast of about 2 feet in depth. The area of these beds is about 1.75 acres, and they give a satisfactory effluent compared with the brook when filled with storm water. The Farm is underdrained with agricultural drain pipes, which are laid at depths varying between 4 and 6 feet.

In the case of the Walton Farm, this takes at the present time the sewage of about 35,000 inhabitants spread over an area of 1,650 acres. It has an acreage of 183 acres. There is only one outfall sewer to this Farm, the internal dimensions of which are 3 ft. 6 in. by 2 ft. 4 in. It has a gradient of 1 in 1,300. This sewer discharges through a 38-inch and also an 18-inch pipe into a tank elevated some 6 or 8 feet above the highest point of the Farm. From this tank underground pipe carriers are laid to the different parts of the Farm, where they terminate in chambers provided with penstocks, and the sewage is distributed from these chambers into the various parts of the Farm which require the irrigation.

No special provision is made on this Farm for dealing with storm water, which must all be accommodated upon the surface of the land. This Farm, like the West Derby Farm, is extensively underdrained, and these drains also are collected in the main effluent drains, which discharge into a natural watercourse.

Both the West Derby and the Walton Farms are used for the purpose of raising crops, principally ryegrass, cabbages, potatoes, mangel-wurzels, and turnips.

WEST DERBY FARM

Dry weather flow per 24 hours :—

	Gallons	Total
High-level sewer... ...	725,000	976,000
Low-level sewer	251,000	
Walton Farm	660,000	

STORM WATER PLOT & BEDS—WEST DERBY SEWAGE FARM

In April, 1896, the then City Engineer obtained authority from the Works Committee to form banks about 3 feet high round plot L, which is about 20 acres in area and reasonably level. The object of this was to impound storm water from the western outfall sewer, and to gradually run it off into the 'Alt' through a special coke screening chamber, which was constructed on the intake to the 24-inch pipes which provided the outlet to the 'Alt.' This arrangement was much improved subsequently by providing a 15-inch pipe at a higher level than the 24-inch outlet pipes from the screening chamber; and this 15-inch pipe discharged on to the surface of a burned ballast filter bed about $\frac{1}{4}$ acre in extent, and thence into the 'Alt.' A much clearer effluent was thus obtained than through the coke screens only.

A considerable amount of sludge was deposited all over plot L, although there was only 1 part of sewage in each 36 of storm water, and, as no crops could be raised on any part of this plot, authority was obtained in January, 1897, to form three sludge beds, each rather over 1 acre in area, which should discharge alternately into a common channel and through the coke screening chamber. The effect of these beds was to concentrate the sludge, and to enable a paying crop to be raised on half of the remaining area of plot L. The other half remainder (about 8 acres) was to be devoted to storage of storm water. These two plots of about 8 acres have been changed about each year, being one year under cultivation and next year under water.

The cost of the sludge beds was about £250

BACTERIOLOGICAL FILTER BEDS

In the year 1898 the Corporation of Liverpool decided to put down filter beds, with the object of assisting the irrigation area of the West Derby Sewage Farm. The original proposal was to construct four coarse and four fine beds of a cheap construction, the excavations being out of the solid clay, and the floors and sides being faced with concrete. Owing, however, to difficulties in connection with levels, and other local conditions, the number of beds was reduced to six in three series. The first contact beds in each series had also to be built above the natural surface of the ground, the secondary beds only being in the solid ground. The beds in use, and which comprise three systems, are numbered 1, 1*a*, 2, 2*a*, 3, 3*a*.

BEDS Nos. 1 AND 1*a*.

In this pair, the first contact bed (No. 1) was made up of burnt clay to a depth of about 2 ft. 8 in., and the second contact bed (No. 1*a*) has burnt clay in the bottom to a depth of about 14 inches, and on the top of this coke broken to about the size of the ballast, making a total depth of 2 ft. 8 in.

Bed No. 1 has an area of 230 square yards, and No. 1*a* an area of 254 square yards, with a capacity of 205 and 226 cubic yards respectively. The area of the two beds combined equals one-tenth acre.

These beds commenced working on the 28th June, 1899, and were charged with crude sewage three times per 24 hours, each charge being allowed to remain in contact with the material in the bed for at least two hours. This rate of working was maintained up to the 25th October, 1899, without any period of absolute rest, with the exception of an occasional charge or two which were missed in times of heavy rainfall. On the 25th October, owing to the surface of Bed No. 1 becoming very foul, the charges were reduced to one per day, with the result that an improvement in the surface began at once to be noticeable. At the present time the charges on these beds are two per day, the increase taking place from the 13th December, 1899.

On the 23rd January, 1900, a section of the bed was exposed and little if any sludge was found in the bottom ; but the whole of the material was uniformly and somewhat heavily coated with dark-coloured sewage matter, and worms were found in considerable numbers. The beds opened up sweet, and apparently without any bad smell whatever. Owing to the action of the weather upon the clay ballast, it was necessary on the 16th March, 1900, to remove about 3 inches from the surface and to replace it with coke.

During the whole period the second contact bed No. 1*a* had worked well in conjunction with No. 1, as regards quantities, and has kept very clean on the surface.

The following statement shows the capacity of the bed at the various times at which it has been tested by measurement :—

Date	Cubic feet	Gallons
*June 28th, 1899	2,900	18,120
July 6th, „	2,500	15,620
October 6th, „	2,050	12,810
November 29th, „	1,900	11,870
December 13th, „	1,850	11,560
January 23rd, 1900	1,760	10,940
February 23rd, „	1,700	10,620
March 19th, „	1,600	10,000
April 23rd, „	1,500	9,370

*On this date, which was the first time on which the bed was charged, the material was dry.

BEDS Nos. 2 AND 2*a*

The first contact bed (No. 2) is made up of red sandstone obtained from Wavertree, and has a depth of about 3 feet. Bed No. 2*a* is constructed of coke broken to about the size of coke breeze.

The area of bed No. 2 is 230 square yards and of No. 2*a* 254 square yards, with a capacity of 230 cubic yards and 254 cubic yards respectively.

These beds commenced working on the 17th August, 1899, and were charged, as in the case of No. 1, with three charges per day, the only relief being during times of heavy rainfall, when the sewers were delivering practically nothing but storm water. This rate of working was reduced to two charges per day on the 24th February, 1900, owing to the surface of the bed showing signs of foulness, and this reduced rate at present continues.

On the 23rd January, 1900, the bed was opened up, and about 1½ inches of sludge was found at the bottom, but in other respects it was in a similar condition to No. 1 bed.

A noticeable feature in the case of this bed was the length of time the sandstone remained clean and bright upon the surface.

The following statement shows the capacity of the bed at the various times on which it has been tested by measurement :—

Date	Cubic feet	Gallons
* August 17th, 1899	2,450	15,310
„ 18th, „	2,300	14,370
October 6th, „	2,150	13,440
November 29th, „	2,100	13,120
December 19th, „	2,050	12,810
January 24th, 1900	1,950	12,190
February 23rd, „	1,900	11,870
March 19th, „	1,800	11,250
April 23rd, „	1,700	10,620

BEDS Nos. 3 AND 3*a*.

In this instance the first contact bed is composed of ordinary gas coke placed in position as carted from the works, and has a depth of about 3 feet.

The second contact bed (No. 3*a*) is filled with sand and gravel, the greater part consisting of sand containing a certain amount of grit. This is, however, imported into Liverpool as Wyre Gravel.

* First time of charging, material dry.

As in the case of the other beds, the combined area of the bed is one-tenth of an acre ; No. 3 bed being 230 square yards in extent, and No. 3*a* 254 yards, with a capacity of 230 cubic yards and 254 yards respectively. Owing to the nature of the material in 3*a*, it has been found that the area of the sand bed should be twice that of the coke bed.

These beds commenced working on November 14th, 1899, at the rate of one charge per day through No. 3, this being equal to filling 3*a* twice. On November 20th the rate was increased to two fillings of No. 3, or four for No. 3*a*, which was continued until the 16th January, 1900, when the rate was reduced to one charge for No. 3, owing to the difficulty in getting the water through the sand. As the rate of working No. 3*a* did not improve, about 2 inches in depth of the surface was removed, and on restarting a great improvement was noticed, which, however, only continued for 12 or 14 days, the surface becoming gradually clogged up again. The operation of removing the surface of the bed has had to be repeated on two occasions, and digging over the surface has also been tried, the result in each case being a temporary improvement.

Both beds were opened up for examination at the same time as Nos. 1 and 2. No sludge was found in the bottom of No. 3, and no worms were to be seen, but a slight covering of sewage matter was on the material of the bed, and large patches of a reddish-brown colour, resembling iron-rust, were noticed, especially near the surface. No. 3*a* was opened carefully to the bottom, and showed signs of clogging for about 3 inches from the surface, below which the material appeared as fresh as when put in.

The following statement shows the capacity of No. 3 bed at the various times on which it has been tested by measurement :—

Date	Cubic feet	Gallons
*November 14th, 1899	3,500	21,870
„ 16th, „	3,200	20,000
„ 29th, „	3,150	19,690
December 20th, „	2,800	17,500
January 25th, 1900	2,700	16,870
February 23rd, „	2,700	16,870
March 19th, „	2,700	16,870
April 23rd, „	2,700	16,870

The effect produced by the working of the bacteriological beds was most marked, not only on the Sewage Farm but on the purity of the River Alt, into which

* First time of charging, material dry.

all effluent drains from the Farm discharge. It was the subject of general remark that the River Alt had not been so clean for many years.

NOTE.—On May 3rd, 1900, the beds 1, 2, and 3 were opened up. There was a considerable amount of sludge on the surface of the bed 1, and the burnt ballast was throughout uniformly covered with one-eighth inch sludge. On bed 2 there was a considerable amount of sludge on the surface, and the stones were coated to the bottom with half-an-inch of sludge. In bed 3, which was only doing half of the work of the other beds, the material had only a very slight coating of sludge.

BACTERIOLOGICAL INVESTIGATIONS

NUMBER OF BACTERIA PER C.C. PRESENT IN THE CRUDE SEWAGE DISCHARGED AT THE WEST DERBY AND WALTON SEWAGE FARMS

WEST DERBY SEWAGE FARM. EASTERN OUTFALL, LOW LEVEL

Date	Dilution	No. of Plates	Average No. of Bacteria per c.c.
November 7th, 1898	$\frac{1}{10,000}$	6	587,000
	$\frac{1}{100,000}$	5	820,000
„ 11th, „	$\frac{1}{10,000}$	6	3,307,000
	$\frac{1}{100,000}$	6	5,250,000
„ 15th, „	$\frac{1}{100,000}$	6	17,200,000
„ 18th, „	$\frac{1}{100,000}$	3	2,000,000
„ 21st, „	$\frac{1}{100,000}$	6	19,640,000
„ 28th, „	$\frac{1}{10,000}$	4	265,000
	$\frac{1}{100,000}$	5	420,000
June 27th, 1899 ...	$\frac{1}{100,000}$		5,640,000
July 23rd, „ ...	$\frac{1}{100,000}$		650,000
„ 25th, „ ...	$\frac{1}{100,000}$		3,650,000

Western Outfall, High Level

Date	Dilution	No. of Plates	Average No. of Bacteria per c.c.
November 7th, 1898...	1/10,000	6	3,160,000
	1/100,000	6	5,760,000
„ 11th, „	1/10,000	6	32,480,000
	1/100,000	5	35,960,000
„ 15th, „	1/100,000	6	64,600,000
„ 21st, „	1/100,000	6	4,217,000
June 27th, 1899 ...	1/100,000		3,140,000
July 10th, „	1/100,000		6,540,000
„ 13th, „	1/100,000		22,834,000
„ 28th, „	1/100,000		6,000,000
August 23rd, „	1/100,000		3,700,000
September 2nd, „	1/100,000		1,340,000

WALTON SEWAGE FARM

Crude Sewage

Date	Dilution	No. of Plates	Average No. of Bacteria per c.c.
November 11th, 1898	1/10,000	6	9,554,000
	1/100,000	6	32,200,000
„ 15th, „	1/100,000	6	20,800,000
„ 21st, „	1/100,000	6	4,170,000
„ 28th, „	1/100,000	4	425,000

From the above tables it will be seen that the average number of bacteria per c.c. varies very greatly. The average of 29 analyses is 1,090,726.

The Contact Beds

The effluent from the final contact beds, 1*a* and 2*a*, has always contained a considerable amount of matter in suspension, and in large quantity is opaque. The effluent also undergoes slight decomposition on standing. The effluent from bed 3*a* (sand bed) has on the other hand been clear, and has not undergone decomposition on standing, or after incubation. In the case of all three effluents, the following figures show that the total number of organisms, as well as the numbers of the B. coli, are very high.

Table showing the number of bacteria per c.c., the number of B. coli per c.c., and the presence or absence of the B. enteritidis sporogenes in the effluent from bed 2*a* :—

Date	No. of Organisms per c.c.	B. coli per c.c.	B. enteritidis sporogenes in 0.01 c.c.
November 8th, 1899	270,000	11,030	Present
„ 9th, „	325,000	3,475	„
„ 10-11th, „	220,000	21,370	„
„ 13th, „	4,327,000	12,380	„
„ 14th, „	937,000	24,070	„
„ 15th, „	2,060,000	12,770	„
„ 16th, „	2,187,000	7,433	„
„ 17th, „	1,440,000	14,570	„
„ 18th, „	1,284,000	13,000	(in 0.1 c.c.)
„ 27th, „	20,000	100	Present
„ 28th, „	667,000	22,500	„
„ 29th, „	1,196,700		„
„ 30th, „	1,690,000	14,367	„
December 1st, „	840,000	20,100	„
„ 5th, „	2,460,000	11,000	„
„ 7th, „	720,000	1,600	„
„ 8th, „	23,000		
„ 9th, „	13,000	Doubtful if any 200 (?)	„
„ 12th, „	48,000	2,700	„
„ 13th, „	13,700	407	„
„ 14th, „	23,700		
„ 15th, „	7,700	Absent in 1 over 100 c.c.	Absent
„ 16th, „	12,200		„
„ 18th, „	3,870		Present
„ 19th, „	157,200	6,633	„
January 8th, 1900	81,400	2,000	Absent
„ 10th, „	360,000	13,400	„
„ 15th, „	64,470		
„ 19th, „	114,000 41,700		
„ 20th, „	14,700	1,333	
„ 23rd, „	162,000		
„ 24th, „	89,400	14,000	Present
„ 26th, „	91,700	600	
„ 29th, „	119,470		„
„ 30th, „	124,800		

From the above table it will be seen that the average number of bacteria per c.c., as the result of 36 analyses, is 614,158.

The average number of B. coli in 23 analyses is 10,036.

The number of times in which the B. enteritidis sporogenes is present in .01 c.c., in 28 analyses, is 23.

Bacteriological Analyses of the Land Drain Effluent of the West Derby Farm

Of 8 determinations the average number of bacteria per c.c. was 17,887, and the average number of the B. coli 50 per c.c. The B. enteritidis sporogenes was present in the 1 c.c. in 2 out of 5 determinations. It will therefore be seen that the land filtration gives better results than filtration through the artificial beds.

Observations upon the Sand Filter Bed (Bed 3*a*)

As previously stated the effluent from this bed has been very much clearer than those derived from the other 2 contact beds. It has also not undergone decomposition upon incubation. On the other hand bacteriological analyses show that the numbers of B. coli still remains high.

West Derby Sand Bed Effluent

Date	Bacteria	B. coli	B. enteritidis sporogenes
November 28th, 1899 ...		11,400	
December 11th, 1899 ...		26,867	
„ „ „ ...		20,850	

Sand Filter Bed

January 22nd, 1900.—The bed had become clogged by deposit on the surface. An analysis of the effluent gave the following results :—

January 22nd, 1900	Bacteria per c.c.	B. coli per c.c.	B. enteritidis sporogenes
Effluent from Rough Coke Bed running on to Sand...	1,085,000	777	Absent in 0.01 c.c.
Sand Effluent	23,800	533	Present in 1 c.c.
„ after 1 hour...	28,600	663	Absent in 0.01 c.c.

January 23rd.—An analysis of the sand of the bed was made.

January 23rd, 1900	Bacteria per gram.	B. coli	B. enteritidis sporogenes
Top of Bed	34,000,000	33,600	Present in 0.01 gram.
Middle	2,000,000	300	Absent in 0.01 gram.
Bottom	*46,000,000	700	Absent in 0.01 gram.

January 24th, 1900.—The surface of the filter was skimmed off. An analysis of the first filtration gave the following results :—

January 24th, 1900	Bacteria per c.c.	B. coli	B. enteritidis sporogenes
First 10 minutes	75,100	6,100	Absent in 0.8 c.c. (clot)
After 1 hour	63,500	5,900	Absent in 0.9 c.c. (clot)

January 31st, 1900.—Samples of the effluent were taken after the bed had been working for a week.

January 31st, 1900	Bacteria per c.c.	B. coli	B. enteritidis sporogenes in 1 c.c.
Average of Samples taken every ¼ hour for 3 hours...	40,800	6,733	Present
Last of effluent coming from bed	30,300	4,800	Present

Bacteriological Investigations of the Storm Water Filter Beds

On January 22nd, 1900, during a period of heavy rainfall, bacteriological analyses were made of the effluents of the Storm Water Filter Beds.

Source	Bacteria per c.c.	B. coli per c.c.	B. enteritidis sporogenes
Crude Sewage and Storm Water, W. outfall	5,360,000	31,400	Present in 1 c.c.
Crude Sewage and Storm Water, E. outfall	946,700	9,300	
Effluent from Burnt Ballast Filter	149,600	4,133	Absent in .01 c.c.
Effluent from Cinder Bed Filter	290,600	733	Absent in .01 c.c.

* One organism chiefly.

From these figures it will be seen that the storm water filters produce a very considerable bacterial purification. The satisfactory action of these beds is probably explained by the very long periods of rest which they obtain.

Clogging of Filter Beds

A year's constant working having very considerably reduced the capacity of the beds, it has been decided to give them a rest from June 9th, 1900. Before doing so, however, analyses were made on June 9th to show exactly the bacterial condition of the effluents after the one year's working. The following table shows the number of bacteria per c.c., the number of B. coli, and the presence or absence of the B. enteritidis sporogenes. It will be seen that the numbers still remain very high.

Source	Bacteria per c.c.	B. coli per c.c.	B. enteritidis sporogenes .01 c.c.
Bed 1*a*	1,270,000	7,000	Present
Bed 2*a*	837,000	21,100	Absent
Bed 3*a*	740,000	12,000	Present

NOTE UPON THE

TWO SPECIES OF 'FUNGUS' COMMONLY FOUND IN SEWAGE CONTAMINATED WATER

By RUBERT BOYCE

The 'Sewage Fungus' is a gelatinous, cotton wool-like and wavy, white or reddish growth, which is found in shallow running water, covering stones, lining drain pipes, or attached to water plants and débris. Its presence indicates sewage contamination, and it is therefore usually to be found in the drains and streams of sewage farms and sewage works, and in the sewage contaminated brooks of villages. The appearance of the fungus is characteristic, and it cannot be readily mistaken for other growths, such as those produced by Streptothrix and Beggiatoa. It may occur in enormous quantities and produce blocking of drain pipes and small streams; or, as it becomes readily detached, accumulations sometimes occur, which are prone to undergo decomposition and give rise to black putrescent masses.

Great interest attaches to the fungus because it indicates sewage pollution. The growth, however, is not found in crude sewage; it is found in the effluents of sewage farms, resulting from the filtration of the crude sewage through the earth, and in brooks which receive small house drains. It indicates, therefore, a certain degree of pollution. In Germany, owing chiefly to the researches of Mez and Schorler, attention has been drawn to the significance of the 'fungus.' It became apparent that at least two distinct growths were included in the term 'sewage fungus,' one *Leptomitus lacteus*, the other *Sphærotilus natans*, the former present in very slightly contaminated water, the latter in highly polluted water.

Leptomitus lacteus (Agardh)*

This fungus belongs to the family of the Saprolegniaceæ. It forms long wavy tufts, springing from the sides of drains or attached to stones. It is very soft, almost gelatinous, and may form white, rusty, or black masses. The white appearance is the natural colour of the growing filaments, but very soon this colour is replaced by a deposit on the hyphœ of oxide of iron. The rusty colour of the fungus is very characteristic, and is an indication that the stream in which it is found is well oxygenated. If the oxygen is absorbed, as in the interior of masses of the growth, or

* Hildebrand, *Jahrb. für wissensch. Bot.* Bd. vi; Pringsheim, *Jahrb. für wissensch. Bot.* Bd. ii; Cohn, *Jahresber. d. Schles. Gesellsch. für Vaterländ*, Cultur, 1852.

in stagnant water, sulphide of iron is formed, and the fungus assumes a black colour and undergoes putrefactive decomposition.

Microscopic examination shows that it consists of long branching filaments, which are constricted at regular intervals; the branches bud off below the constrictions, and each segment has a very characteristic refractile nucleus. Zoospores are formed in the terminal segments.

Sphærotilus natans

This organism may be readily confounded with the preceding. It forms quite as long wavy masses in the drains and streams as does Leptomitus. It is, however, usually white and cotton wool-like when seen in the water. It is much more gelatinous than Leptomitus. On stones in shallow streams, or coating the sides of drain pipes, it forms low velvety or feather-like growths. Like Leptomitus, it requires oxygen, and grows best in shallow water-courses and where there is plenty of movement. Its presence indicates much greater pollution than does the former organism, and therefore it is of importance to be able to distinguish the two forms. In two cases where bacterial analyses were made of the water in which both forms of 'fungus' were found, that in which Leptomitus occurred contained at least less than 100 Bacillus coli per c.c., whilst that in which Sphærotilus was found contained over 19,000 Bacillus coli per c.c. With the introduction of the bacterial bed method of treatment, this organism has also made its appearance. In one case, where in the method of treatment the bacterial filter is warmed, I found that the Sphærotilus had made its appearance in large quantity, the food materials—circulation, aeration, and warmth—being the conditions most favourable to its development. It is, therefore, an organism which may cause blocking of aerobic contact beds.

Sphærotilus has been long confounded with Beggiatoa. It is allied to the Leptothrix forms. As the microscopic preparations show, it occurs in chains of short bacilli, or as long undivided filaments. It is, therefore, very much more minute than Leptomitus; this is readily seen from the photomicrographs, which are equally magnified. The filaments and rods are surrounded with a gelatinous capsule.

In crude, undiluted sewage, a skin-like growth may form at the sides of the conduit in contact with the air, or at those points where crude sewage passes over a 'lip.' The growth, which has a coarse velvety appearance, consists of club-shaped zooglea masses of bacilli, and it seems probable that this bacterium, if not identical with Sphærotilus, is closely allied to it.

Sphærotilus may also be confounded with Carchesium. I have found *Carchesium Lachmanni* coating the surface of the stones in a polluted stream and producing the white cotton wool-like appearance of the fungus; in fact, on the farm in question, it was always regarded as the 'sewage fungus'; microscopic examination, Plate VI., Fig. 6, showed, however, a striking difference.

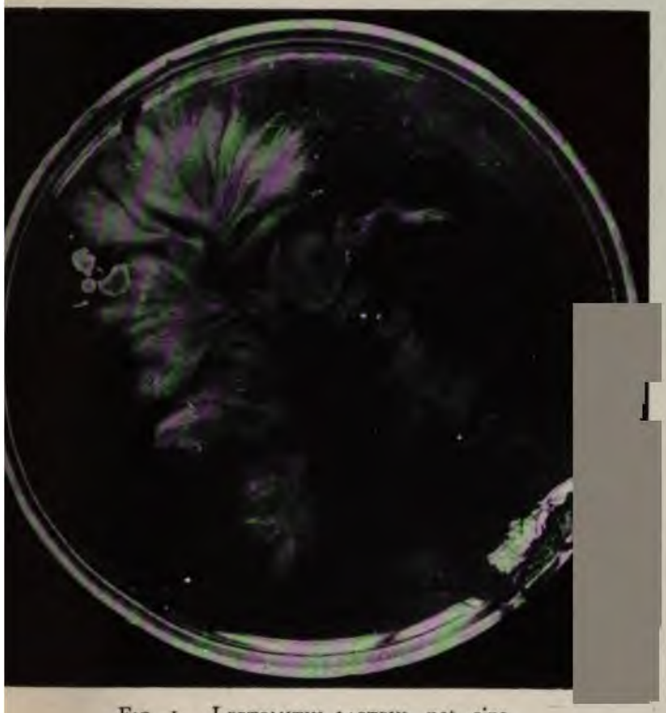


FIG. 1. LEPTOMITUS LACTEUS, nat. size.

FIG. 2. SPHÆROTILUS NATANS, nat. size.

FIG. 3. LEPTOMITUS LACTEUS, showing branching.

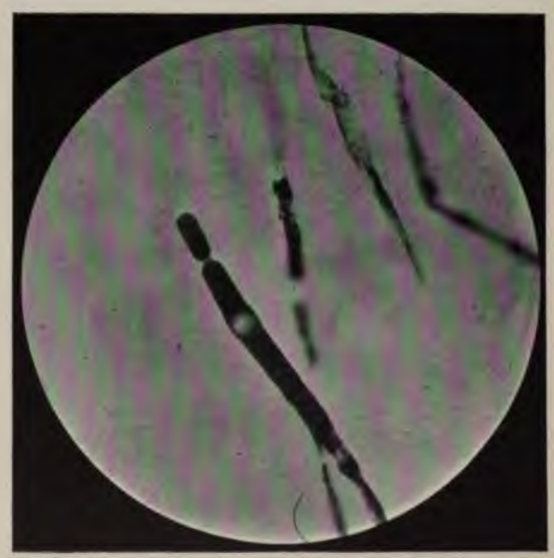


FIG. 4. LEPTOMITUS LACTEUS, showing constriction and nucleus.

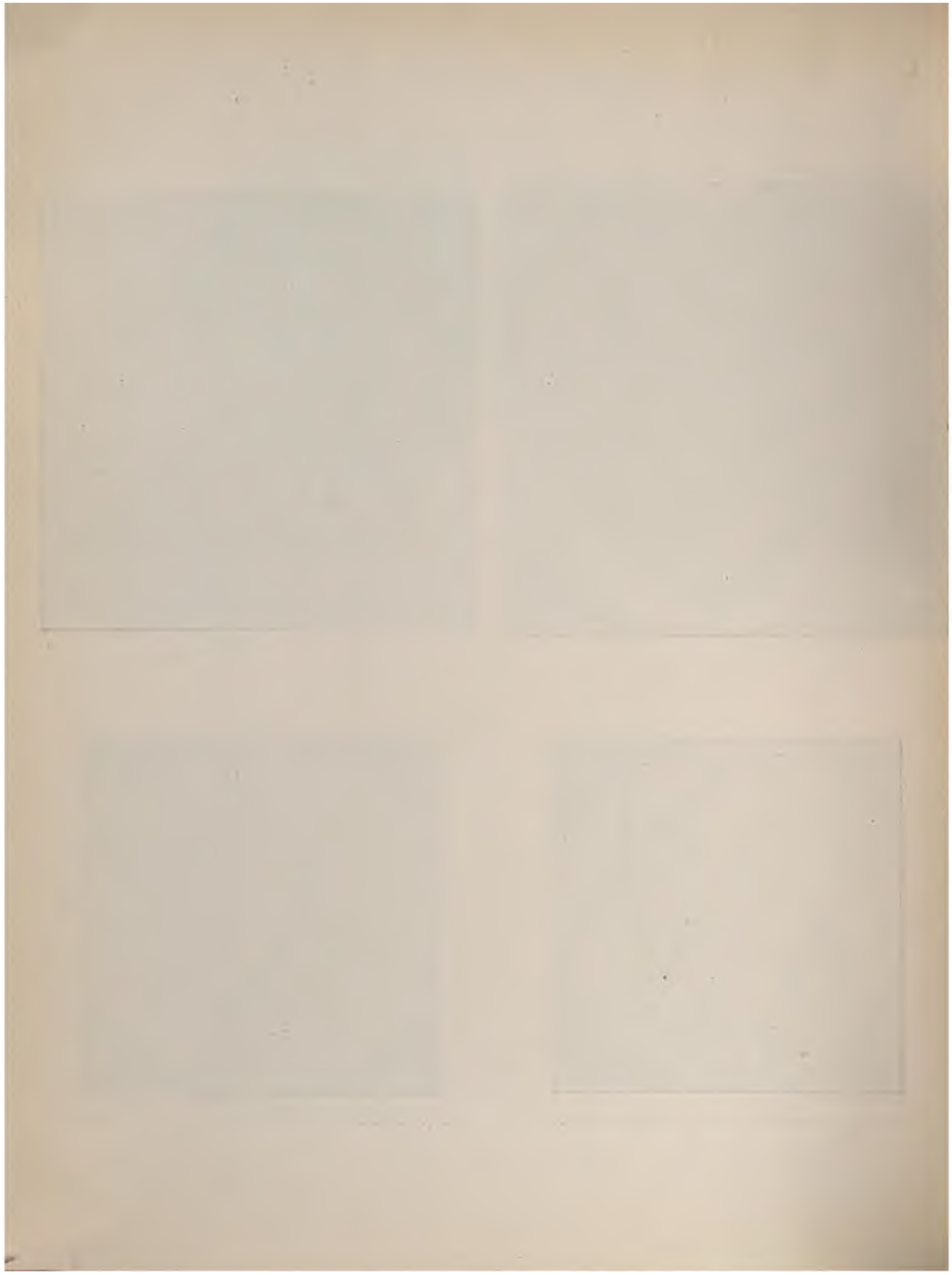


FIG 5. SPHÆROTILUS NATANS.

FIG. 6. CARCHESIUM LACHMANNI.

Leptomitus, Sphærotilus, the zooglea forming bacteria, and Carchesium Lachmanni, all indicate a water which contains special food stuffs derived from sewage; they also indicate aeration. Leptomitus indicates the least amount of contamination and the greatest amount of aeration; the zooglea masses are present in concentrated sewage, and Sphærotilus and Carchesium hold intermediate places.

These organisms perform a very important function—they follow up and complete the work of the bacteria in breaking up organic matter; they are succeeded by higher forms of ~~animal~~ life—the Diatomaceæ and the Green Algæ, whilst these in their turn give place to the fishes and green plants. The 'sewage fungus,' therefore, plays a very considerable share in the self-purification of water.

PRESERVATIVES AND COLOURING MATTERS IN FOODS

By E. W. HOPE, M.D., D.Sc.

The importance of avoiding waste in regard to food stuffs of any kind does not need to be emphasized, but it is of greater importance to insure that the means taken to prevent waste are not calculated to injure health.

The preservation of meat and other perishable foods by means of cold is, as is well known, largely resorted to in this country. The successful application of cold as a preservative receives its best illustration in the case of the Copenhagen milk supply, into the details of which it is not now necessary to enter.

In this country the use of chemical preservatives is exceedingly common, and in the case of some very perishable articles, such as milk and cream, it is to be feared, takes the place of care and cleanliness.

It is established beyond dispute that chemical preservatives, whilst checking putrefactive changes in the food, may also check the fermentative processes of digestion.

Boracic acid or borates, as a preservative, is found in margarine, butter, ham, bacon, pork, fish, cream, and milk. In margarine and butter the use of it has been common for many years; it is usually more or less uniformly mixed with these articles, and about 30 grains to the pound have been found. It is also met with in sausages, pork pies, and pastry. An article sold as 'Arcticanus Special Cream Compound' is a mixture of boracic acid and borax.

Salicylic acid and salicylates are met with in jam, but the manufacturers appear to be bonâ fide anxious to minimise the quantity. In the case of British wines, however, as well as in the case of some unfermented drinks, sometimes both boracic acid and salicylic acid have been found, and there are not evidences of the same care on the part of the manufacturers to limit the amount, the quantity varying from 7 grains to 150 grains of salicylic acid to the gallon, and 4 grains to 100 grains of boracic acid to the gallon.

It is perfectly plain that if it is absolutely necessary to add either of these drugs, and if 7 grains to the gallon is enough in one case, there is no necessity to throw in 150 grains to the gallon in another case.

In prosecutions which have been undertaken in regard to British wines, the defendants have actually had the effrontery to put forward the defence that the chemicals employed were useful as drugs, and they had actually induced medical men

to go into the witness-box to prove to the court the value of these drugs when used medicinally, the evidence proving nothing more than that a dose of physic can be administered without injury in certain diseased conditions of the human frame; but the promiscuous administration of doses of physic of this character at meal times is known to have a very prejudicial effect, especially upon young infants.

The addition of formalin, or boracic acid, to milk, appears to be exceedingly uncommon in Liverpool, a growing tendency last year to the use of these materials in milk having been checked by a prosecution under the Food and Drugs Act.

It is abundantly plain that there can be no real necessity for the use of these drugs in milk; if it were a real necessity the sale of milk could not go on as it does, without their use. Of all articles in which the use of chemical preservatives is likely to be attended with mischief, milk is the most likely, and it is in this case that the use of preservatives is most indefensible. The experiments conducted in the Bacteriological Department of the THOMPSON YATES LABORATORIES are sufficient to establish the dangers of the practice, even if they stood alone. There are numerous cases of injury resulting from the use of milk so preserved. One may be quoted:— Mrs. E. T. voluntarily offered her milk dealer twice the price he asked to send to her pure milk to be used for her infant. The double price, however, did not deter the dealer on one occasion from sending milk containing boracic acid preservative, the presence of which was immediately detected by its effect upon the child. The dealer made no secret of his action when taxed with it. In cases such as this the use of the preservative is distinctly toxic, hence, if the use be allowed by law, the fact should be made known to the purchaser, since of all casual dangers to health, those arising from the use of poisons in food are the most difficult to defend one's self from.

COLOURING MATTERS

Colouring matters seem to have two distinct uses in foods—in the one case, merely to give an attractive appearance to an article, but without imitating any other article; and in the other case, it is added to increase the resemblance of the article to that which it is intended to simulate.

The commonest colouring matter, which is usually used for sausages, especially German sausages, consists of a mixture of borax, red coal tar dye of the class known as sulphonated diazol, with a little salt or saltpetre, and sometimes ground rice or bread crumbs mixed with it. Armenian bole consists of oxide of iron with a siliceous matter.

The use of these colouring matters by no means necessarily implies fraudulent intent—purchasers might be supposed to know that no natural food stuff could have the remarkable colour of some German sausages; but there is another colouring matter which might, without injustice, be suspected of bordering upon the fraudulent; that is 'Smokene.' This is a mixture of borax, salt, creosote, and red coal tar dye,

which is used for brushing over hams, bacon, tongue, fish, &c., and which gives the article the appearance of having been perfectly and carefully smoked, the operation, like the name, being very ingenious. Amongst other common fraudulent colourings may be mentioned the use of burnt sugar with dilute acetic acid to resemble vinegar, and the use of glucose with a very common black treacle to imitate the brightness of golden syrup.

The dirt, or staleness of goods may be concealed by colouring matters, as, for example, dirty rice, used to make egg powders, coloured with a yellow coal tar dye which takes away the dirty appearance; similarly, stale milk coloured with a slightly yellow dye, gets a richer look. With regard to egg powders, which in reality are merely baking powders, these are coloured yellow and labelled 'each packet equivalent to one egg.' Cases have actually come under notice in which the purchaser has believed that the packet did actually contain the equivalent in food of a desiccated egg, possibly the yellow colour completed the delusion.

It does not appear that the Food and Drugs Act, which is essentially framed on commercial lines, discountenances the use of colouring matters and preservatives, unless it can be proved that such ingredients are injurious to health.

Every person adding any colouring matter, or preservative whatever, to articles of food, should state on a plain, simple and conspicuous label—

1. The material used.
2. The quantity used.
3. The date at which the material was added.

A form of label for supplying this information might be suggested.

Heat and cold as preservatives are exceedingly common, and many articles are now sold as sterile. An article sold as sterile ought certainly to be sterile; if it is not, it possesses the disadvantage of giving a false sense of security to the purchaser. But the sterility should not be secured by using chemicals.

In procuring samples with a view to obtaining information as to the extent to which any given preservative, colouring matter, or adulterant is used, it is of the utmost importance that persons trained in the procedure of obtaining samples should be employed; they, of course, can make use of the services of agents when necessary, but a case may be instanced in which a gentleman not conversant with the methods obtained a considerable number of samples, and on causing them to be analysed found all of them to contain the same preservative. Inquiry, however, showed that all these samples had been supplied from one and the same source.

Preservatives and Cold

Last year chemical preservatives were substituted for cold to a very great extent in the case of imported pork. This pork is packed in small boxes of 50 lbs. each, which are placed in a refrigerator on board ship with other 'chilled' meat, and

kept at a temperature of 32 or 35 to 38 degrees. Previous to packing it is treated in some way with boracic acid, which finds its way into the flesh. The reason for putting it in is to check accidental decomposition, which would result from any defect in the cooling process. If pork is frozen it spoils the appearance and deteriorates the value; hence it can only be 'chilled.'

As illustrating the sudden increase of this trade last year, although the number of pigs slaughtered in the City was well maintained—in fact, was considerably in excess of the previous year—the imports amounted to no less than 29,000 more than in the preceding year, the total 36,227 being nearly five times as large as it was in the preceding year, when the trade appears to have commenced. Prior to that the carcases of dead pigs imported did not exceed a few dozen annually, the trade being practically nil.

Another important aspect of the question of chemical preservatives arises in connection with the increasing demand for, and supply of, sterilized milk, more especially for infants, all over the country.

Many of the firms supplying sterilized milk are not milk-producing firms, the milk being supplied by contract from wholesale milk dealers.

It is obvious that if the milk dealers have added chemical preservatives to the milk sent to the sterilizing companies, the greatest danger to the health of infants will be incurred. It is not reasonable to expect that the sterilizing companies will systematically, day by day, cause analyses to be made to ascertain the presence of these preservatives; but their use should be altogether prohibited, and cleanliness and cold relied upon to ensure preservation of the milk.

The following special samples of home and imported goods were submitted during 1899 for special examination, in order to determine the character and amount of the preservative and colouring matter used :—

SUMMARY

Bacon	5	Meat Juice...	1
Brawn	2	Pork Pie	2
Cheese	6	Port Wine	3
Chicken Broth	1	Arcticanus	1
Claret	1	Armenian Bole	1
Cocoa and Milk	1	Bi-Sulphite of Lime	1
Condensed Milk	2	Indian Red	1
Fruit Jelly	1	Meat Preservative	1
Ham	5	Poloney Dye	1
Jams	7	Rose Pink	1
Kidney Soup	1	Sausage Colouring	1
Meat Jelly	1	Smokene	1

REPORT TO THE MEDICAL OFFICER OF HEALTH OF THE INVESTIGATIONS AND ANALYSES MADE BY THE CORPORATION BACTERIOLOGIST

The work of the Bacteriologist comprises :—

(*a*) Examination of food stuffs of various kinds.

(*b*) Regular examination of water supplied to the City.

(*c*) Examinations into suspected cases of rabies, anthrax, glanders, &c.

(*d*) Examination for diagnostic purposes in suspected cases of diphtheria, typhoid fever, tubercular sputum, &c.

(*e*) Special investigations.

In the examination of food stuffs the following general methods are employed :—

Samples of milk and samples of water are collected in sterilized bottles, and if they cannot be examined immediately they are kept in a refrigerating chamber.

(1) *Preservatives in Food.* In view of the increasing use of preservatives of various kinds in food stuffs, and the difficulty which has been experienced in proving in Courts of Law the limit beyond which it was dangerous to extend their use, the Medical Officer requested the Bacteriologist to undertake some exact experiments into the physiological action of boracic acid and formalin, as these, especially the latter, were found in foods likely to be used for infants and young children.

A series of experiments were conducted by Dr. ANNETT, which consisted in feeding kittens of three weeks old upon milk containing minute but definite proportions of the preservatives in question, and the observations extended in each case over a period of from six to seven weeks. With regard to the boracic acid, (*a*) Five kittens were fed upon milk containing 10 grains to the pint ; (*b*) Five kittens were fed upon milk containing 5 grains to the pint ; (*c*) Five kittens were fed upon perfectly pure milk, the kind of food being the only difference in the treatment of the animals. In every case a supply of milk was always available for the kittens to lap.

Results. The group (*a*) fed with 10 grains of boracic acid to the pint showed rapid emaciation, diarrhœa, and death resulted in from three to four weeks ; the kittens treated with 5 grains showed results almost similar. The remaining five kittens fed with pure milk increased in weight and remained healthy.

With regard to the second series of milk, viz., that treated with formalin, the proportions of formalin, which is a very powerful antiseptic, were used as follows :— Five kittens were fed with milk containing 1 part in 50,000 of milk, five with 1 in 25,000 of milk, and five with 1 in 12,500. As in the preceding cases, there was a

continuous supply for the kittens to lap. Four kittens were fed at the same time with wholesome milk.

The results showed (*a*) the five kittens fed with the weakest solution (1 in 50,000) showed an average increase of weight per week equal only to 70 per cent. of the healthily fed kittens; those fed with the next strongest solution (*b*) (1 in 25,000) increased in weight only to 55 per cent. of that of the healthily fed kittens; (*c*) those animals fed with the proportion containing the largest amount of formalin, namely, 1 in 12,500, increased in weight only from 20 to 25 per cent. of the amount which the healthily fed kittens increased. Two of the number died after six weeks, suffering from emaciation, diarrhœa, and other evidence of disturbed digestion and starvation. The whole of the animals were kept under careful observation, and it appeared that the dosed animals showed general evidences of ill-health, inactivity, and so on, and a disinclination to feed on the preserved milks.

There is not the slightest reason to doubt that the use for infants of milk or cream containing these preservatives will have precisely the same effects upon the infants as they have upon kittens, and the inquiry once more emphasizes the absolute necessity for feeding infants as nature intended they should be fed, or if that is impracticable, to employ pure and natural substitutes.

Formalin, fortunately, is not largely used by milk dealers as a preservative adulterant. About 1,500 samples of milk were chemically examined for the specific purpose of ascertaining if formalin was present, but it was only found in .8 per cent. of these samples.

(2) *Anthrax in Tanneries.* Several cases of Anthrax having occurred during 1899 amongst the workers of the tan-yards, four of which proved fatal, a thorough investigation of the vats, hides, and dust of the tan-yards was made by the Bacteriologist and Dr. GRÜNBAUM. The bacillus anthracis was found in large quantities in the gloves which the workmen used whilst cleaning the hides. The bacillus was also found in the vats used for macerating the hides, and in the dust of the yards.

It was evident that the Anthrax was introduced from the hides taken from infected animals, and a special investigation was then made of the ships which brought over the hides from Eastern ports. This resulted in finding the anthrax bacillus in the dust of a hold from a ship which had contained bales of hides. Subsequently an investigation was made to ascertain the kind of disinfectant which could be relied upon to destroy the anthrax bacilli (which is one most tenacious of life), but without injuring the hides.

(3) *The Tuberculin Reaction.* Some cows which reacted to tuberculin were slaughtered, and the subsequent inoculations proved the presence of the tubercle bacillus, although the lesions were very small, and confined to minute nodules in the lungs and bronchial glands.

(4) *Sewage Investigations.* The importance of bacteria in the treatment and disposal of sewage matter has occupied much attention recently. A large proportion of the work of the Royal Commission on Sewage Disposal is done at the THOMPSON YATES LABORATORIES, and the Health Committee authorized a very large number of experiments to be carried out at the Sewage Farms at West Derby. These investigations have been very useful. The effluents from the Sewage Farm drains have been carefully examined from time to time, and have shown a very good average purification. Experimental filter beds were constructed out of various materials, and have now been at work many months. The effluents yielded by some of the beds have been very good, showing a considerable amount of purification.

(5) *Plague Investigations.* During the summer, rats taken from ships in which plague had occurred were carefully investigated for the presence of the plague bacillus. They proved to be free. Two cases of Bubo, also from suspected ships, were examined for the presence of the plague bacillus, but careful bacteriological examination of the glands clearly demonstrated that the swelling was not due to this cause. The bacteriological diagnosis in these cases proved of the greatest importance in demonstrating the nature of the diseases suspected to be plague. The Assistant Corporation Bacteriologist (Dr. BALFOUR STEWART) has published during the year several papers dealing with his researches upon plague.

(6) *Fish Poisoning.* A bacteriological investigation into the cases of fish poisoning resulted in the discovery of organisms kindred to those found in the putrefying contents of infants' feeding bottles. It is very difficult to say whether the organisms alone are capable of producing the irritant symptoms, or whether these are due to some product resulting from the growth of the bacilli. Be it as it may, in either case the importance of absolute cleanliness is emphasized by the investigation.

RABIES

During the year there have been no cases of Rabies, but eight cases of suspected Rabies, mostly reported by the police, have been carefully investigated; in none of the cases could the presence of that disease be ascertained.

The Medical Officer has arranged with the Board of Agriculture that in cases of suspected Rabies the inoculation test shall be made by the Corporation Bacteriologists, which obviates the necessity for sending parts of the bodies of dogs suspected of having that disease to London.

THE BACTERIOLOGICAL DIAGNOSIS OF CASES OF TYPHOID AND DIPHTHERIA

The total number of diagnostic examinations made for the City hospitals was 201. It is of the greatest importance to be able to state whether the organism which causes Diphtheria is present in the throat or not. With regard to the bacteriological diagnosis of Typhoid Fever, evidence is accumulating of the value of the test.

Pathological Diagnosis Society

This society is formed by a number of medical men, who desire diagnostic investigations to be carried out in respect to patients under their care. It is an extremely useful society, and numbers about 200 medical men.

The work of this society supplements that done for the Corporation. For an annual subscription of 10s. 6d. any practitioner can place any number of cases of Diphtheria, Typhoid, Tubercle, and other infective processes, urine and tumours for bacteriological examination. The number of investigations in this connection has nearly reached 1,000 during the year.

SPECIAL INVESTIGATIONS

Summer Diarrhœa

The Medical Officer requested the Bacteriologist to make investigations into the condition of the artificial foods supplied to infants during the season of exceptional mortality from summer diarrhœa.

The homes of the poor where sickness was known to exist were visited, and the feeding bottles found in use, together with their contents, were removed to the laboratories, new bottles being given in their place at the cost of the Health Committee. Furthermore, the same practice was adopted at the dispensaries, the feeding bottles actually in use, and their contents, being taken away for examination, and other bottles given to replace them.

The bacteriological examination confirmed the evidence of the senses, the putrefying and offensive contents of the bottles containing innumerable putrefactive organisms, amongst them being the bacillus enteritidis sporogenes, which, judging from the effects of the injection of bacilli of this description into Guinea-pigs, is virulently destructive of life.

The tubes connected with the bottles were also found to contain putrefactive matter. The examination added confirmation to the neglect, ignorance, and carelessness of the parents.

Cases of Food Poisoning

On Thursday, September 28th, 1899, the Deputy Coroner held inquests on five persons who died from acute food poisoning.

The facts shortly are as follows :—The deceased persons, all women between 44 and 70 years of age, had partaken of either cooked salt fish or pig's cheek at the stall of Mrs. B——, in St. Martin's Market, on the 14th, 15th, or 16th of September. In a few hours after eating the food each person became seriously ill with symptoms of acute food poisoning, and eventually died, four of them the day after taking the food, and the other the next day but one.

After consultation with the Medical Officer, a post-mortem and bacteriological examination was ordered by the Coroner to be made in each case by the Corporation Bacteriologist.

The result of the examination showed that in all the cases the naked eye appearances were identical, and indicated a very rapid and intense infection. In three cases the Bacteriologist was immediately able to discover the presence of the bacillus enteritidis sporogenes, and in the other two, although this bacillus was not definitely proved to exist at the date of inquest, there appeared strong evidence that it was or had been present.

The bacillus enteritidis sporogenes is found in sewage, foul air and water, foul vessels and filthy clothes, and in decomposing horse manure, &c. It is very resistant to heat, and its spores will survive the action of boiling water for some time.

The Bacteriologist has also recently found it in dirty feeding bottles of infants suffering from acute diarrhœa.

In the case of the persons who died after eating food in St. Martin's Market, there was no evidence to prove how the food became contaminated by the bacillus. It may have been in the food before cooking, though in the case of the salt fish that is unlikely, or it may have infected the food afterwards. Inquiry showed that the food was kept under insanitary conditions by the vendor, at her house, and by alternate wetting and drying, cooking, and carrying through the streets, it would be in a condition suitable for the growth and development of the bacillus.

The Bacteriologist carried out experiments with portions of similar cooked and uncooked food, and with scrapings of stalls, &c., on which it lay. No examination of the actual offending food in these cases was possible, as the deceased persons ate their portions in the market, and by the time their deaths were known the stall holder had no further sample of the same lot left. She, in fact, stated that she never carried over any cooked food from day to day.

Frequent observations by the Fish Inspectors have failed to show any naked eye appearances of diseased food on the stall.

Besides the five fatal cases, three other cases of illness came under the notice of the Medical Officer. These persons ate some of the same food in company with one or other of the deceased persons, and afterwards suffered with serious symptoms of food poisoning.

A table giving particulars of all the cases is appended. It is probable that a considerable number of persons ate food from Mrs. B——'s stall on the dates in question without suffering any ill effects.

Name	Age	Date of Illness	Result of Illness	Nature of Food	Bacteriologist's Report
Catherine K... ...	48	Sept. 14th...	Death, Sept. 15th...	Pig's Cheek...	No Bacillus Enteritidis Sporogenes
Teresa D.	44	„ 15th...	„ „ 16th...	Fish	No Bacillus Enteritidis Sporogenes
Ellen M.	54	„ 15th...	„ „ 17th...	„	Bacillus Enteritidis Sporogenes found
Margaret McG. ...	66-70	„ 16th...	„ „ 17th...	„	Bacillus Enteritidis Sporogenes found
Ann G.	62	„ 16th...	„ „ 17th...	Fish and Pea Soup ...	Bacillus Enteritidis Sporogenes found
Charles D.	15	„ 15th...	Recovery	Fish	
Catherine C.... ...	—	„ 15th...	„	„	
Esther K.	—	„ 16th...	„	Fish and Pea Soup ...	

Samples taken for Bacteriological Analysis or Examination

Anchovy Paste	... ...	1	
Bloater „	... ...	2	
Bovril ...	... ...	1	
Camp Coffee	... ...	1	
Cheese	... ...	6	
Chicken, Ham, and Tongue	...	3	
Cockles	... ...	4	
Crab ...	... ...	1	
Cream ...	... ...	2	
Desiccated Egg Food	...	1	
„ Soup	... ...	1	
Dry Antiseptic	... ...	1	
Fruit Juice Lemonade	...	1	
Ham and Tongue (Tinned)	...	1	
Ham and Chicken	... ...	1	
Ice ...	... ...	1	
Jams ...	... ...	17	
Jellies ...	... ...	4	
Ketchup	... ...	1	
Lemon Cheese	... ...	1	
Lobster (Tinned)	... ...	1	
Malted Milk	... ...	1	
Margarine	... ...	14	
Marmalade	... ...	5	
Milk ...	... ...	353	
„ Condensed	... ...	4	
Mussels	... ...	5	
Olive Oil	... ...	1	
Oysters	... ...	4	
Periwinkles	... ...	1	
Potted Beef	... ...	2	
„ Game	... ...	1	
„ Ham	... ...	3	
„ Shrimps	... ..	3	
„ Tongue	... ...	2	
„ Venison	... ...	1	
„ Lobster	... ...	2	
Preserved Peas	... ...	1	
„ Peaches	... ...	1	
„ Pears	... ...	1	
„ Pineapple...	...	1	
„ Tomatoes	...	1	
Salmon (Tinned)	... ...	7	
Salted Haddock	... ...	6	
„ Cod	... ...	8	
„ Ling	... ...	2	
Sardines (Tinned)	... ...	9	
„ (Smoked, Tinned)	...	1	
Sausages (Smoked)	... ...	1	
Sauces ...	... ...	4	
Sterilized Milk	... ...	5	
Turkey and Tongue (Tinned)	...	2	
Veal and Ham	... ...	2	
Total	... ...	506	

Milk.—The total number of samples analyzed has been 352. Of these 159 have been taken at the railway stations on arrival from the country, and 162 from the town.

Of the 159 railway samples, in 27 cases the inoculated animals died before the observation was completed, and of the remaining 132, 15 were proved to be tubercular, giving the percentage of tubercle in railway-borne milk as 11.3 per cent.

Of the town milks, 23 inoculated animals died before the observation was completed; of the remainder 1 was tubercular, thus giving a percentage of .7, or less than 1 per cent.

There were 31 examinations of special samples of milk. None of these were tubercular.

In the following table the + sign denoted the presence of tubercle, the — sign the absence of tubercle, and the word negative indicates that the animal died before the tubercular reaction had time to develop, if it were present in the milk which was inoculated.

MILKS

No.	Source	Date	Tubercle present or absent
450	Town	January 4th	—
451	Town	January 4th	Negative
455	Rail	January 6th	—
456	Rail	January 6th	Negative
459	Town	January 12th	—
460	Town	January 12th	—
461	Town	January 12th	—
462	Town	January 12th	—
467	Rail	January 17th	—
468	Rail	January 17th	—
469	Rail	January 17th	+
470	Rail	January 17th	+
471	Town	January 17th	—
475	Rail	January 25th	—
476	Rail	January 25th	—
477	Rail	January 25th	—
478	Rail	January 25th	—
479	Town	January 26th	—
480	Town	January 26th	—
484	Town	January 30th	—
485	Town	January 31st	—
486	Town	January 31st	—
487	Town	January 31st	—
488	Town	January 31st	—
492	Rail	February 8th	+
493	Rail	February 8th	+
494	Rail	February 8th	—
495	Rail	February 8th	—
496	Rail	February 10th	—
503	Town	February 16th	—

MILKS—*continued*

No.	Source	Date	Tubercle present or absent
504	Town	February 16th	—
505	Town	February 16th	—
507	Town	February 16th	—
508	Rail	February 23rd	—
509	Rail	February 23rd	—
510	Rail	February 23rd	—
511	Rail	February 23rd	—
512	Rail	February 23rd	—
519	Rail	March 2nd	—
520	Rail	March 2nd	—
521	Rail	March 2nd	Negative
522	Rail	March 2nd	—
523	Rail	March 2nd	—
524	Rail	March 9th	—
525	Rail	March 9th	—
526	Rail	March 9th	—
527	Rail	March 9th	—
528	Rail	March 9th	—
532	Town	March 6th	—
533	Town	March 6th	—
534	Town	March 6th	—
535	Town	March 6th	—
536	Town	March 6th	—
548	Town	March 29th	—
549	Town	March 29th	—
550	Town	March 29th	—
551	Town	March 29th	Negative
552	Town	March 29th	—
540	Rail	March 23rd	—
541	Rail	March 23rd	—
542	Rail	March 23rd	—
543	Rail	March 23rd	—
544	Rail	March 23rd	—
561	Rail	April 7th	+
562	Rail	April 7th	+
563	Rail	April 7th	—
564	Rail	April 7th	—
565	Rail	April 7th	—
569	Town	April 13th	—
570	Town	April 13th	—
571	Town	April 13th	—
572	Town	April 13th	—
573	Town	April 13th	—
580	Rail	April 21st	—
581	Rail	April 21st	+
582	Rail	April 21st	+
583	Rail	April 21st	+
584	Rail	April 21st	—
588	Town	April 27th	Negative
589	Town	April 27th	—
590	Town	April 27th	—

MILKS—*continued*

No.	Source	Date	Tubercle present or absent
591	Town	April 27th	—
592	Town	April 27th	—
593	Rail	May 4th	—
594	Rail	May 4th	—
595	Rail	May 4th	Negative
596	Rail	May 4th	Negative
597	Rail	May 4th	Negative
601	Town	May 11th	—
602	Town	May 11th	Negative
603	Town	May 11th	—
604	Town	May 11th	Negative
605	Town	May 11th	—
610	Park Style Farm	May 16th	—
611	P.S.F.	May 16th	—
612	P.S.F.	May 16th	—
613	P.S.F.	May 16th	—
614	P.S.F.	May 16th	—
615	P.S.F.	May 16th	—
616	P.S.F.	May 16th	—
617	P.S.F.	May 16th	—
618	P.S.F.	May 16th	—
619	P.S.F.	May 20th	Negative
620	P.S.F.	May 20th	Negative
621	P.S.F.	May 20th	—
622	P.S.F.	May 20th	—
623	P.S.F.	May 20th	—
624	P.S.F.	May 20th	—
625	P.S.F.	May 20th	—
626	P.S.F.	May 20th	—
627	P.S.F.	May 20th	—
630	Rail	May 26th	—
631	Rail	May 26th	Negative
632	Rail	May 26th	—
633	Rail	May 26th	—
634	Rail	May 26th	—
635	Town	June 2nd	—
636	Town	June 2nd	—
637	Town	June 2nd	—
638	Town	June 2nd	—
639	Town	June 2nd	—
643	Rail	June 6th	—
644	Rail	June 6th	—
645	Rail	June 6th	—
646	Rail	June 6th	Negative
647	Rail	June 6th	—
651	Girls' Indus. Sch.	June 14th	—
652	Seamen's Orphan.	June 15th	—
653	Town	June 15th	—
654	Town	June 15th	Negative
659	Rail	June 22nd	—
660	Rail	June 22nd	—

MILKS—*continued*

No.	Source	Date	Tubercle present or absent
661	Rail	June 22nd	—
662	Rail	June 22nd	—
663	Rail	June 22nd	—
669	Town	June 29th	—
670	Town	June 29th	—
671	Town	June 29th	—
672	Town	June 29th	—
673	Town	June 29th	—
676	Rail	July 6th	—
677	Rail	July 6th	—
678	Rail	July 6th	—
679	Rail	July 6th	Negative
680	Rail	July 7th	—
683	Town	July 14th	Negative
684	Town	July 14th	—
685	Town	July 14th	—
686	Town	July 14th	—
687	Town	July 14th	—
691	Rail	July 20th	—
692	Rail	July 20th	—
693	Rail	July 20th	—
694	Rail	July 20th	Negative
695	Rail	July 20th	—
698	Special	July 21st	—
701	Town	July 27th	Negative
702	Town	July 27th	—
703	Town	July 27th	—
704	Town	July 27th	—
705	Town	July 27th	—
707	Country	July 31st	Negative
708	Country	July 31st	—
709	Country	July 31st	—
710	Country	July 31st	—
711	Country	July 31st	Negative
712	Town	August 2nd	+
713	Town	August 1st	—
714	Town	August 1st	—
715	Town	August 2nd	—
716	Town	August 2nd	—
717	Town	August 3rd	—
718	Town	August 3rd	—
719	Country	August 3rd	Negative
720	Country	August 3rd	—
721	Rail	August 10th	—
722	Rail	August 10th	—
723	Rail	August 10th	—
724	Rail	August 10th	—
725	Rail	August 10th	—
726	Town	August 17th	—
727	Town	August 17th	—
728	Town	August 17th	—

MILKS—*continued*

No.	Source	Date	Tubercle present or absent
729	Town	August 17th	—
730	Town	August 17th	—
735	Rail	August 26th	—
736	Rail	August 21st	—
737	Rail	August 26th	—
738	Rail	August 26th	—
739	Rail	August 26th	—
740	Town	August 30th	—
741	Town	August 31st	—
742	Town	August 30th	—
743	Town	August 30th	Negative
744	Town	August 30th	—
748	Rail	September 7th	—
749	Rail	September 7th	—
750	Rail	September 7th	Negative
751	Rail	September 7th	—
752	Rail	September 7th	—
759	Town	September 15th	—
760	Town	September 15th	Negative
761	Town	September 15th	—
762	Town	September 15th	—
763	Town	September 15th	—
764	Rail	September 21st	—
765	Rail	September 21st	—
766	Rail	September 21st	—
767	Rail	September 21st	—
768	Rail	September 21st	—
772	Town	September 28th	—
773	Town	September 28th	—
774	Town	September 28th	—
775	Town	September 24th	—
776	Town	September 28th	—
780	Netherfield Road Hospital	October 2nd	—
781	Mill Lane Hospital	October 3rd	—
782	Priory Rd. Hospital	October 4th	—
783	Town	October 4th	—
787	Town	October 5th	—
788	Rail	October 6th	—
789	Rail	October 6th	+
790	Rail	October 6th	—
791	Rail	October 6th	+
792	Rail	October 6th	—
793	Town	October 12th	—
794	Town	October 12th	—
795	Town	October 12th	—
796	Town	October 12th	—
797	Town	October 12th	—
798	Town	October 12th	—
799	Town	October 12th	—
800	Town	October 12th	—

MILKS—*continued*

No.	Source	Date	Tubercle present or absent
801	Town	October 12th	Negative
802	Town	October 19th	—
809	Rail	October 19th	Negative
810	Rail	October 19th	—
811	Rail	October 19th	—
812	Rail	October 19th	—
813	Rail	October 19th	Negative
814	Rail	October 19th	—
815	Rail	October 19th	—
816	Rail	October 19th	—
817	Rail	October 19th	—
818	Rail	October 19th	—
819	Town	October 27th	—
820	Town	October 27th	—
821	Town	October 27th	—
822	Town	October 27th	—
823	Town	October 27th	—
824	Town	October 27th	—
825	Town	October 27th	—
826	Town	October 27th	—
827	Town	October 27th	—
828	Town	October 27th	—
833	Rail	November 3rd	—
834	Rail	November 3rd	—
835	Rail	November 3rd	—
836	Rail	November 3rd	—
837	Rail	November 3rd	Negative
838	Rail	November 3rd	—
839	Rail	November 3rd	Negative
840	Rail	November 3rd	—
841	Rail	November 3rd	Negative
842	Rail	November 3rd	Negative
843	Town	November 9th	—
844	Town	November 9th	—
845	Town	November 9th	Negative
846	Town	November 9th	—
847	Town	November 9th	Negative
848	Town	November 9th	—
849	Town	November 9th	—
850	Town	November 9th	—
851	Town	November 9th	—
852	Town	November 9th	—
855	Rail	November 14th	Negative
856	Rail	November 14th	—
857	Rail	November 14th	—
858	Rail	November 14th	—
859	Rail	November 14th	Negative
860	Rail	November 14th	—
861	Rail	November 14th	—
863	Town	November 14th	Negative
864	Rail	November 16th	Negative

MILKS—*continued*

No.	Source	Date	Tubercle present or absent
865	Rail	November 16th	—
871	Town	November 24th	—
872	Town	November 24th	—
873	Town	November 24th	—
874	Town	November 24th	—
875	Town	November 24th	—
876	Town	November 24th	Negative
877	Town	November 24th	—
878	Town	November 24th	—
880	Town	November 24th	Negative
881	Town	November 24th	—
882	Town	November 28th	—
883	Town	November 29th	Negative
884	Town	November 29th	—
885	Rail	November 30th	—
886	Rail	November 30th	—
887	Rail	November 30th	—
888	Rail	November 30th	—
889	Rail	November 30th	—
890	Rail	November 30th	Negative
891	Rail	November 30th	—
892	Rail	November 30th	—
893	Rail	November 30th	—
894	Rail	December 1st	Negative
895	Town	December 1st	—
896	Town	December 6th	—
897	Town	December 6th	—
898	Town	December 6th	Negative
899	Town	December 6th	—
900	Town	December 6th	Negative
901	Town	December 6th	—
902	Town	December 6th	—
903	Town	December 6th	—
904	Town	December 6th	Negative
905	Town	December 6th	—
906	Rail	December 14th	Negative
907	Rail	December 14th	+
908	Rail	December 14th	—
909	Rail	December 14th	Negative
910	Rail	December 14th	—
911	Rail	December 14th	Negative
912	Rail	December 14th	—
913	Rail	December 14th	—
914	Rail	December 14th	Negative
915	Rail	December 14th	—
918	Town	December 19th	—
920	Town	December 20th	—
921	Town	December 20th	—
922	Town	December 20th	Negative
925	Town	December 21st	—
926	Town	December 21st	—

MILKS—*continued*

No.	Source	Date	Tubercle present or absent
927	Town	December 21st	—
928	Town	December 21st	Negative
930	Rail	December 29th	—
931	Rail	December 29th	—
932	Rail	December 29th	—
933	Rail	December 29th	Negative
934	Rail	December 29th	+
935	Rail	December 29th	+
936	Rail	December 29th	Negative
937	Rail	December 29th	—
938	Rail	December 29th	+

General Conclusions.—It will be seen from the analysis of milk that the occurrence of tubercle in the milk obtained from the shippons in the city is diminishing. The presence of the bacillus tuberculosis in the railway-borne milk is, however, still too frequent, although there is no doubt that many of the milk dealers realize the importance, in their own interests, of banishing tubercle.

Preserved Milks, Cheese, Butter, and Margarine

Condensed Milks.—As in the previous year, the condensed tinned milks are not always found to be sterile, or free from organisms. This form of milk ought always to be sterile; if it is not it has no advantage over raw milk, from a bacteriological point of view, and it may be the means of distributing pathogenic germs.

Sterilized Milks.—The analyses have revealed the presence of bacteria. This is most unsatisfactory, and shows that sufficient care is not taken to sterilize the milk, and that the manufacturers do not employ proper means to ensure the efficacy of their sterilizing processes, nor do they adopt tests to prove that their methods are effectual. Unless milk sold as sterilized is actually so it has no advantage over non-sterilized milk; on the contrary, the consumers are entirely misled, and an article is supplied which is not of the nature, substance, and quality demanded.

TINNED MILKS

No.	Date	Result
517	February 28th	Sterile
628	May 24th	Not Sterile
831	November 2nd	No B. Coli Communis No B. Enteritidis Sporogenes
832	November 2nd	No B. Enteritidis Sporogenes No B. Coli Communis

MALTED MILKS

No.	Date	Result
518	February 28th	Sterile

CREAM

No.	Date	Result
699	July 23rd	Non-tubercular
771	July 21st	Non-tubercular

STERILIZED MILK

No.	Date	Name	Result
700	July 26th	Sterilized Milk	Not Sterile
731	August 24th	Sterilized Humanized Milk	Not Sterile
732	August 24th	Sterilized Humanized Milk	Not Sterile
733	August 24th	Sterilized Milk	Not Sterile
734	August 24th	Sterilized Milk	Not Sterile

MARGARINE

No.	Date	Result
500	February 14th	No Tubercle
501	February 14th	No Tubercle
502	February 14th	No Tubercle
528	March 10th	No Tubercle
535	March 29th	No Tubercle
556	March 29th	No Tubercle
559	April 5th	No Tubercle
560	April 5th	No Tubercle
574	April 14th	Non-tubercular
578	April 20th	No Tubercle
579	April 20th	Suspicion of Tubercle, Doubtful
608	May 12th	No Tubercle
830	October 27th	No Tubercle

CHEESE

No.	Date	Result
553	March 29th	Non-tubercular
554	March 29th	Non-tubercular
575	April 14th	Non-tubercular
606	May 12th	Non-tubercular
607	May 12th	Non-tubercular
809	October 27th	Non-tubercular No Enteritidis Sporogenes

Jams, etc.—A large number of samples have been examined and no pathogenic bacteria found. A very considerable number of the jams are sterile, a condition which is very satisfactory, the result of careful preparation. But the use of preservatives for this purpose, by lessening digestibility, lessens or destroys the nutritious properties of the food.

JAMS, ETC.

No.	Date	Name	Result
489	February 3rd	Strawberry	Spores Mould Fungi
490	February 3rd	Black Currant	Sterile
491	February 3rd	Raspberry	Sterile
513	February 23rd	Marmalade	Sterile
585	April 26th	Gooseberry and Apple	Sterile
586	April 26th	Damson and Apple	Sterile

SHELL-FISH

No.	Date	Name	Result
452	January 5th	Oysters	No B. Coli Communis
453	January 5th	Oysters	No B. Coli Communis
463	January 12th	Mussels	No B. Coli Communis
481	January 27th	Cockles	No B. Coli Communis
482	January 27th	Mussels	No B. Coli Communis
483	January 27th	Oysters	No B. Coli Communis
537	March 17th	Mussels	No B. Coli Communis
538	March 17th	Cockles	No B. Coli Communis
805	October 18th	Oysters	No B. Enteritidis Sporogenes
806	October 18th	Mussels	No B. Enteritidis Sporogenes
807	October 18th	Cockles	No B. Enteritidis Sporogenes
808	October 18th	Periwinkles	No B. Enteritidis Sporogenes
923	December 21st	Cockles	No B. Enteritidis Sporogenes No B. Coli Communis
924	December 21st	Mussels	No B. Enteritidis Sporogenes No B. Coli Communis

SAUCES

No.	Date	Name	Result
617	June 28th	Mint Sauce	Not Sterile
668	June 28th	Chutney	Not Sterile
784	October 6th	Chutney	Not Sterile
803	October 13th	Worcester Sauce	Sterile

MISCELLANEOUS

No.	Date	Name	Result
479	February 10th	Desiccated Soup	Not Sterile
514	February 23rd	Desiccated Egg Food	Numerous Organisms
539	March 17th	Crab	Not Sterile
629	May 24th	Ice	Numerous Organisms
777	September 29th	Camp Coffee	Not Sterile
785	October 5th	Lemon Cheese	Not Sterile
786	October 5th	Olive Oil	Sterile

WATER ANALYSES

During the year 1899 systematic examinations of the drinking water have been made. In collecting the samples the usual precautions to prevent extraneous contamination have been taken. The analyses show that the water is uniformly very good. No pathogenic forms have been isolated, and the average number of bacteria per cubic centimetre has been well below 100 (KOCH's Standard). A very large number of analyses have also been made for the purposes of the Water Committee, and these appear in the separate report of the Water Committee.

The following are the sources which have been examined :—

FORTNIGHTLY EXAMINATIONS

Ashton Hall Tap

MONTHLY EXAMINATIONS

PRESCOT— { Lake Vyrnwy Water, Rivington Water, The Mixed Water } WELLS— { Windsor Well, Dudlow Lane Well, Green Lane Well }

ASHTON HALL—FORTNIGHTLY SAMPLES

Source	Date	Time of collecting	Time of investment	Bacteria present Gelatine	Agar
Ashton Hall	January 7th	10 am.	10 5 am.	25	3
,,	January 20th	2 25 pm.	2 30 pm.	120	50
,,	February 6th	2 pm.	2 7 pm.	104	11
,,	February 15th	10 15 am.	10 25 am.	21	12
,,	March 2nd	4 pm.	4 15 pm.	89	21
,,	March 20th	12 noon	12 7 pm.	68	15
,,	April 1st	10 10 am.	10 15 pm.	87	26
,,	April 17th	2 pm.	2 7 pm.	1550	240
,,	April 21st	10 5 am.	10 9 am.	143	67
,,	May 12th	5 pm.	5 7 pm.	9	2
,,	May 26th	4 pm.	4 7 pm.	63	10
,,	June 10th	10 am.	10 7 pm.	31	5
,,	June 21st	4 pm.	4 5 pm.	75	16
,,	July 5th	2 pm.	2 7 pm.	77	22
,,	July 19th	2 pm.	2 8 pm.	81	—
,,	August 19th	—	—	100	—
,,	August 29th	2 35 pm.	2 45 pm.	88	96
,,	September 15th	9 45 am.	9 54 am.	50	—
,,	September 27th	2 30 pm.	2 45 pm.	19	17
,,	October 10th	11 30 am.	11 45 am.	30	—
,,	October 30th	9 am.	9 5 am.	40	10
,,	November 10th	9 30 am.	10 am.	15	12
,,	November 25th	1 pm.	5 30 pm.	Liquefied	—
,,	December 7th	10 am.	10 10 am.	24	12
,,	December 21st	2 30 pm.	2 40 pm.	23	—

PRESCOT—VYRNWY WATER—MONTHLY SAMPLES

Source	Date	Time of collecting	Time of investment	Bacteria present Gelatine	Agar
Vyrnwy	January 17th	12 30 pm.	1 pm.	52	16
,,	February	—	—	—	—
,,	March 29th	11 am.	12 30 pm.	112	128
,,	April 26th	2 pm.	3 30 pm.	83	4
,,	May 15th	—	3 35 pm.	13	8
,,	June 21st	2 30 pm.	4 10 pm.	58	13
,,	July 17th	2 15 pm.	4 pm.	43	—
,,	August 28th	2 pm.	4 pm.	24	48
,,	September 29th	2 2 pm.	3 30 pm.	8	13
,,	October 27th	12 30 pm.	4 pm.	30	—
,,	November 24th	12 45 pm.	3 30 pm.	10	7
,,	December 20th	2 5 pm.	3 45 pm.	15	—

PRESCOT—RIVINGTON WATER—Monthly Samples

Source	Date	Time of collecting	Time of investment	Bacteria present Gelatine	Agar
Rivington	March 1st	11 am.	12 noon	15	170
,,	March 29th	11 am.	12 30 pm.	104	368
,,	April 26th	2 pm.	3 30 pm.	22	15
,,	May 15th	2 15 pm.	3 35 pm.	21	11
,,	June 21st	2 30 pm.	4 10 pm	70	20
,,	July 17th	2 17 pm.	4 pm.	25	—
,,	August 28th	2 5 pm.	4 pm.	2	24
,,	September 29th	2 7 pm.	3 30 pm.	30	3
,,	October 27th	12 35 pm.	4 pm.	30	8
,,	November 24th	12 50 pm.	3 pm.	9	—
,,	December 20th	2 8 pm.	3 45 pm.	20	—

PRESCOT—MIXING WELL—Monthly Samples

Source	Date	Time of collecting	Time of investment	Bacteria present Gelatine	Agar
Mixing Well	January 17th	12 30 pm.	1 pm.	50	23
,,	February	—	—	—	—
,,	March 29th	11 am.	12 30 pm.	40	43
,,	April 26th	2 pm.	3 30 pm.	51	73
,,	May 15th	2 15 pm.	3 35 pm.	53	200
,,	June 21st	2 30 pm.	4 10 pm.	120	47
,,	July 17th	2 20 pm.	—	107	—
,,	August 28th	2 10 pm.	4 pm.	72	80
,,	September 29th	2 10 pm.	3 30 pm.	88	—
,,	October 27th	12 40 pm.	4 pm.	50	45
,,	November 24th	12 55 pm.	3 pm.	Liquefied	3
,,	December 20th	2 10 pm.	4 pm.	10	—

WINDSOR WELL—Monthly Samples

Source	Date	Time of collecting	Time of investment	Bacteria present Gelatine	Agar
Windsor Well	January 20th	2 15 pm.	2 30 pm.	105	6
,,	March 2nd	8 40 am.	9 5 am.	93	31
,,	March 30th	8 35 am.	9 20 pm.	39	37
,,	April 29th	8 30 am.	9 am.	114	38
,,	May 26th	9 am.	9 20 am.	400	57
,,	June 20th	2 pm.	2 20 pm.	12	8
,,	July 19th	8 15 am.	9 am.	28	—
,,	August 29th	10 42 am.	11 20 am.	10	11
,,	September 27th	9 35 am.	2 pm.	22	5
,,	October 26th	2 30 pm.	11 am. Oct. 27	70	—
,,	November 25th	11 am.	5 30 pm.	44	2
,,	December 19th	3 30 pm.	4 pm.	80	4

DUDLOW LANE—Monthly Samples

Source	Date	Time of collecting	Time of investment	Bacteria present Gelatine	Agar
Dudlow Lane	January	—	—	—	—
,,	February	—	—	—	—
,,	March 1st	2 48 pm.	4 10 pm.	16	8
,,	March 29th	2 45 pm.	4 15 pm.	12	7
,,	April 28th	10 am.	11 45 am.	31	22

DUDLOW LANE—MONTHLY SAMPLES—*continued*

Source	Date	Time of collecting	Time of investment	Bacteria present Gelatine	Agar
Dudlow Lane	May 26th	3 pm.	4 20 pm.	350	41
,,	June 21st	10 4 am.	12 50 pm.	6	3
,,	July 18th	2 30 pm.	4 pm.	20	—
,,	August 28th	11 15 am.	2 pm.	72	56
,,	September 27th	10 20 am.	2 pm.	10	10
,,	October 26th	12 15 pm.	11 am. Oct. 27	30	—
,,	November 25th	11 30 am.	5 30 pm.	152	—
,,	December 19th	3 10 pm.	4 pm.	80	—
Woolton—Tap in Engine Room	January 18th	3 pm.	3 30 pm.	28	4
Reservoir	January 18th	3 pm.	3 30 pm.	320	13
,,	February	—	—	—	—
,,	March 1st	2 40 pm.	4 10 pm.	20	13
,,	March 29th	3 10 pm.	4 15 pm.	14	12

GREEN LANE WELLS—MONTHLY SAMPLES

Source	Date	Time of collecting	Time of investment	Bacteria present Gelatine	Agar
G. Holt	January	—	—	—	—
,,	February	—	—	—	—
,,	March 1st	3 30 pm.	4 10 pm.	35	3
,,	March 29th	3 3 pm.	4 15 pm.	26	24
,,	April 28th	11 am.	11 45 am.	Colonies too numerous to be counted	
,,	May 26th	2 pm.	4 15 pm.	104	70
,,	June 21st	11 30 am.	12 50 am.	200	500
,,	July 18th	3 30 pm.	4 pm.	640	—
,,	August 28th	10 45 am.	2 pm.	296	512
,,	September 27th	11 17 am.	2 pm.	17	12
,,	October 26th	1 5 pm.	11 am. Oct. 27	240	40
,,	November	—	—	—	—
,,	December 19th	1 45 pm.	4 pm.	85	—
J. Holmes	January	—	—	—	—
,,	February	—	—	—	—
,,	March 1st	3 33 pm.	4 10 pm.	20	340
,,	March 29th	3 35 pm.	4 15 pm	73	64
,,	April 28th	11 am.	11 45 am.	Colonies too numerous to be counted	
,,	May 26th	2 10 pm.	4 15 pm.	112	160
,,	June	—	—	—	—
,,	July 18th	3 30 pm.	4 pm.	364	—
,,	August 28th	10 45 am.	2 pm.	Liquefied	—
,,	September 27th	11 21 am.	2 pm.	37	340
,,	October 26th	1 pm.	11 am. Oct. 27	50	3200
,,	November 25th	12 noon	5 30 pm.	240	—
,,	December 19th	1 45 pm.	4 pm.	94	—

The following gentlemen have assisted in the bacteriological work :—Dr. BALFOUR STEWART, Assistant Bacteriologist; Dr. GRÜNBAUM, Dr. WARRINGTON, Dr. ANNETT, and Dr. HILL.

THE DISTRIBUTION OF TUBERCULOSIS IN LIVERPOOL

J. H. ELLIOTT, M.B. (Tor.)

Considering the great importance of the study of the distribution of Tuberculosis in our large cities, I have taken advantage of the opportunity afforded me by a short residence in Liverpool to make a few notes regarding the occurrence of the disease throughout the city.

The incorporated area of the city is 13,236 acres, over 700 of which are occupied by docks and quays. The estimated population in 1898 was 668,645 ; the figures given by the last census were 629,443. This population of 50.5 to the acre is only exceeded in Great Britain by London, West Ham, and Glasgow, while Brighton, Bolton, and Edinburgh have about the same number per acre.

The city is divided into 11 Wards, the population of these varying from 9.8 per acre in Wavertree to 160.4 per acre in Everton. Four of the Wards—Walton, West Derby (rural), Wavertree, and Toxteth (rural) were added to the city in 1895. They are practically suburban; Walton, with 29.8 per acre, being the most densely populated. The remaining Wards have a population of over 100 per acre, with the exception of Abercromby and Exchange ; but considering the areas in these Wards, occupied by large retail shops, warehouses, municipal and other public buildings, the actual population per acre, in the part occupied by householders, will be much above the stated figures.

The following table shows the population per acre for the various Wards, as estimated by the Medical Officer of Health for the years 1895-1898 inclusive :—

TABLE I

Population per acre	1895	1896	1897	1898
Scotland	122.3	119.0	115.5	128.0
Exchange	85.8	82.5	99.5	89.2
Abercromby	76.0	74.4	73.3	75.1
Everton	159.7	159.7	159.7	160.4
Kirkdale	96.0	96.4	96.7	102.8
West Derby	117.1	117.5	117.8	125.3
Toxteth	117.8 134.5	123.5	125.4 173.4	124.6
Walton	27.4	29.1	30.7	29.8
West Derby (rural)	12.9	13.1	13.2	14.0
Wavertree	8.2	8.4	8.6	9.8
Toxteth (rural)	21.9	23.5	25.1	25.2

The districts of Walton, West Derby (rural), Wavertree, and Toxteth (rural), having been added in 1895, I have in these notes only made observations on the statistics published since then, so as to be enabled to make comparisons of the various districts. The reports available previous to 1895 cover only the 7 remaining districts, an area of 5,210 acres instead of the 13,236 as at present, and having an average population of 113 per acre.

Also, previous to 1894, the deaths occurring in public institutions were not referred to the districts from which the patient was admitted, as is now done. The statistics for 1899 were not available for incorporation with those of the previous four years.

The following table shows the death rate per 1,000, from all causes, for the four years 1895-1898 inclusive :—

TABLE II

District	Death Rate per 1,000 population				Average of these rates
	1895	1896	1897	1898	
Scotland	38.4	34.5	37.7	36.4	36.7
Exchange	41.2	37.2	39.8	36.9	38.8
Abercromby	27.0	22.4	24.6	24.5	24.6
Everton	27.6	24.3	25.9	24.5	25.6
Kirkdale	24.4	19.5	22.1	18.8	21.2
Wes Derby	24.3	21.4	22.9	21.1	22.4
Toxteth...		20.8	22.9	21.3	22.2
Walton	15.7	12.7	12.9	12.9	13.5
West Derby (rural) ...	17.7	17.5	17.0	16.3	17.1
Wavertree	16.2	14.6	16.4	16.3	16.0
Toxteth (rural)	10.4	9.8	10.2	9.9	10.1

We find the death rate to be greatest in Scotland and Exchange Wards, where, in the parts which are occupied by dwelling-houses, the people are very much crowded, and even the simpler rules of hygiene are much neglected. The death rate here, too, is increased by the fact of there being many lodging-houses in the two districts which are the temporary abodes of the floating population incident to a large city, and increased in Liverpool by its immense shipping. The death rate is markedly less in the less congested districts.

An idea of the class of people in parts of the city is conveyed by the fact that of 1,002 deaths from Tuberculosis in Exchange Ward, from 1895 to 1899, 563 occurred in the Workhouse and Infirmary, and only 439 in the patients' houses.

The death rate per 1,000 population from Tuberculosis in the various districts is as follows :—

TABLE III

District	Death Rate per 1,000 from Tuberculosis				Average of these rates
	1895	1896	1897	1898	
Scotland	3.68	3.91	3.90	3.51	3.75
Exchange	5.36	5.21	4.48	4.39	4.86
Abercromby	3.00	2.84	2.81	2.64	2.82
Everton...	2.55	2.69	2.43	2.86	2.63
Kirkdale	2.77	1.94	2.00	1.91	2.15
West Derby	3.13	2.78	2.88	1.81	2.65
Toxteth	2.11	2.16	2.22	1.94	2.11
Walton...	1.76	1.45	1.42	1.33	1.49
West Derby (rural) ...	1.76	1.74	1.62	1.54	1.64
Wavertree	2.51	1.42	1.52	1.54	1.75
Toxteth (rural)	1.12	1.25	1.46	1.22	1.26

The death rate from Tuberculosis in Scotland is seen to be 3 times as great as in Toxteth (rural), while in Exchange the rate is nearly 4 times as great. In Abercromby, Everton, and West Derby, which are also crowded districts, it is more than twice as great. This bears out our generally accepted belief that Tuberculosis is a disease which is fostered by and varies with the density of the population and the general hygiene observed by the inhabitants of the district. But, on studying Table II, it is seen that the general death rate per thousand is more than 3 times as great in Scotland as in Toxteth (rural), and in Exchange almost 4 times as great; while in Abercromby, Everton, and West Derby it is more than twice as great. So that the same influences which increase Tuberculosis in the crowded districts are also active agents in the increase of other diseases, and in about the same proportion. This appeared to me to be unusual. Although we expect the general death rate to be greater in the poorer parts, and with it the Tuberculosis death rate also increased, we would be inclined to look for more than a proportionate increase in the deaths from Tuberculosis.

To judge more fairly the occurrence of the disease in the various Wards I prepared the following table, showing what percentage of deaths is due to Tuberculosis:

TABLE IV

District	Population per acre, 1898	Deaths from Tuberculosis—Percentage of all Deaths				Average of these
		1895	1896	1897	1898	
Scotland	128.0	9.5	11.3	10.3	9.1	10.0
Exchange	89.0	12.9	14.0	11.2	11.2	12.3
Abercromby	75.1	11.1	12.6	11.4	10.7	11.4
Everton	160.4	9.2	11.0	9.4	11.2	10.2
Kirkdale	102.8	11.3	10.0	9.0	10.2	10.1
West Derby	125.3	12.8	13.0	12.5	9.1	11.9
Toxteth	124.6	8.8	10.4	9.7	9.1	9.5
Walton	29.8	11.8	11.3	10.9	10.3	11.1
West Derby (rural) ...	14.0	10.0	10.8	9.4	9.5	9.9
Wavertree	9.8	15.5	10.2	9.3	9.4	11.1
Toxteth (rural)	25.2	10.9	12.7	8.9	12.3	11.2

Percentage for the whole city for the four years 10.93.

With an average throughout the city for the four years of 10.93 deaths in each 100, it is seen that of the 5 Wards with the greatest population, each having over 100 to the acre, 4 fall below the average; while of 4 Wards with the least number to the acre, a population of from 9.8 to 29.8 per acre, 3 are above the average.

This is, perhaps, only a coincidence, and should not carry much weight in localizing Tuberculosis in one district rather than another, but it is certainly interesting.

However, it is remarkable that throughout the city, with Wards varying in density of population, and in the general attention paid to personal and general household hygiene, the number of deaths in every 100 due to Tuberculosis should be so uniform—all lying between 9.5 and 12.3. Although in individual years it has varied more than this, as in 1895, from 8.5 in Toxteth to 15.5 in Wavertree. In 1896 the rate was generally high, the lowest rate being 10.0 and the highest 14.0; while in 1898 the rate was generally low, with the exception of 1 Ward, and only 3 Wards had a mortality greater than 10.7.

The great number of deaths each year reported as due to Bronchitis makes it doubtful if these figures are to be entirely relied upon. Were these cases of Bronchitis more carefully diagnosed it is very probable many of them would be cases of Pulmonary Tuberculosis. In 1898 there were 402 more deaths from Bronchitis than from Phthisis and Tuberculosis; there being 1,808 from the former, and 1,406 from the latter. Of these cases, there occurred in the various institutions 534 cases of Phthisis

and Tuberculosis, and 439 of Bronchitis; while in the houses throughout the city there were 872 deaths from Phthisis and Tuberculosis, with 1,349 from Bronchitis, showing a marked preponderance of the latter.

The following table shows the mortality reported under Phthisis and Tuberculosis, as compared with Bronchitis. These are the deaths occurring in the houses throughout the city, and do not include deaths in public institutions:—

TABLE V

	Scotland	Exchange	Abercromby	Everton	Kirkdale	West Derby	Toxteth	Walton	West Derby (rural)	Wavertree	Toxteth (rural)	Total
1898												
Tuberculosis & Phthisis	83	52	62	175	80	148	137	43	44	20	28	872
Bronchitis	162	138	111	251	111	147	258	61	70	23	17	1344
1897												
Tuberculosis & Phthisis	79	53	65	152	84	151	133	51	42	19	31	860
Bronchitis	225	175	117	334	146	147	242	50	68	18	22	1549
1896												
Tuberculosis & Phthisis	62	53	67	142	88	128	125	49	32	16	20	752
Bronchitis	208	137	90	289	142	163	222	44	58	16	23	1392

With the exception of West Derby, Wavertree, and Toxteth (rural), every district shows a marked preponderance of Bronchitis, in fact, so much that it makes one feel very doubtful as to the accuracy of the stated cause of death.

On an ordnance survey map of Exchange District I have marked the houses in which deaths have occurred from Tuberculosis, including deaths in the various Hospitals and Infirmaries of patients admitted from this district, referring the same to the houses from which they were received. Under Tuberculosis I have included all deaths reported under the terms Phthisis, Consumption, Tuberculosis, and Tabes mesenterica. The five years, 1895-1899 inclusive, have been taken. In some few cases, principally deaths in institutions, an incorrect or incomplete address has been given, and it has been impossible to locate these cases. In a few, when the number of the court is given but no house number, a red dot has been placed in the court, simply approximating the locality.

As noted previously, a considerable part of Exchange District is occupied by public buildings, warehouses, and large shops, and in these areas the population is very sparse. Consequently only a portion of the Ward is here reproduced, giving a fair idea of the distribution of the cases throughout other similarly inhabited areas.

What is very striking is the grouping of cases—either two or more in one house, or contiguous houses, with cases occurring in each.

Some of the houses where many cases have occurred are large lodging-houses, *e.g.*, 30 Byrom Street, 131 and 132 Richmond Row, 107 St. Anne Street. In 131 Richmond Row are about 100 lodgers, while 107 St. Anne Street has about 200; many of these are transient, only remaining a few days.

So many cases occurring in the crowded parts, and it being doubtful what precautions are taken in the care of the sputum—and it is known that in a great number of instances practically no care is taken—it is simply incalculable what number of cases receive in such rooms their primary infection.

Although the infective properties of the dust of rooms in which consumptives have slept have been demonstrated over and over again, I asked the Medical Officer of Health in January of this year for a list of houses in which deaths from consumption had recently occurred. Of ten houses in which deaths had occurred 7 to 14 days previously, I was able to secure dust in four. In the others, from being vacant or from objections by the occupants, I was unable to secure any.

The following are my notes at time of taking the dust:—

House A.—Patient slept on lounge 2 to 3 weeks before death. Rooms very clean; difficult to secure dust. Three specimens—No. 1, from lounge between seat and back; No. 2, from cracks in floor at base of wall; No. 3, washings of wall paper above doorway.

House B.—Patient slept in room, rising about 8 a.m., was up each day until day preceding death. House very clean. Sputa all burned. Room thoroughly cleaned after patient's death. Could only secure dust in floor cracks near base of wall, and from washings of wall paper. One specimen. No. 4.

House C.—Patient in room for 3 weeks previous to death. Very careless regarding sputa. House very dirty. One sample collected from mantel, wall paper, and floor at base of wall near where bed stood. No. 5.

House D.—Patient in room for a week only. Sputa slight. Room carefully cleansed. Secured dust from under base-board, and from washings. No. 6.

These six specimens of dust were washed with sterilized water, and the washings injected into Guinea-pigs.

No. 2 died on the day after injection, with an extensive subcutaneous extravasation of limpid serum about the seat of injection. The abdominal muscles also were extensively involved.

No. 4, a big brown male, died four weeks after. The cause of death could not be found; there were no evidences of Tuberculosis.

No. 5 died March 16th, two months after injection, with caseating inguinal glands, in which tubercle bacilli were demonstrated. There were also extensive tubercular involvement of liver and spleen.

Nos. 1, 2, and 6 were apparently quite healthy on March 20th, and showed no enlarged glands.

It would not be right to draw conclusions from these experiments in themselves, but as they bear out the results of many other experiments we may safely do so.

The one house, the dust of which proved infective, was the only one in which there had been carelessness in the disposal of the sputa, and in which ordinary cleanliness had not been observed during and after the patient's death.

It shows what a menace the consumptive's room may be to those who spend any time in it, either during the patient's illness or for some time after. When a case has occurred in a lodging-house or hotel many may become infected by occupying, for a short time only, the room in which the patient lay.

It is satisfactory to know that the death rate throughout England from Tuberculosis has gradually decreased during the past 40 or 50 years with improved sanitation. With proper care in the disposal of the sputa it could be made remarkably less.

It is first essential that the patient understands thoroughly his disease is consumption. A patient who is expectorating, if told by his physician that his lungs are 'weak' or 'slightly affected,' will not see the necessity for destroying his expectoration. The physician should tell him frankly what his disease is, and, at the same time, give him to understand that it is not necessarily fatal, or at any rate not immediately so; that he has good chances for recovery, if in an early stage, and if the physician's advice is carefully followed.

The statement generally accepted is that Tuberculosis, directly or indirectly, causes the death of one in every seven throughout England, and one in every four or five of the working classes. In Liverpool, as we have seen, about one death in every nine is directly ascribed to Tuberculosis. These figures impress us with the necessity of doing everything in our power to control its spread.

In order to reach as many cases as possible, it should be a rule to examine the chest of every case presenting for treatment at the out-patient department of the hospitals and dispensaries, whether medical or surgical. This was begun some time ago in some of the American dispensaries, and so many unsuspected cases were discovered that it is now done as a matter of routine, and is bearing excellent results.

It would be advisable, also, to have a small pamphlet for distribution amongst such cases, instructing them about the disposal of their expectoration. It should be carefully prepared, so as to avoid alarming them or their friends, for if the danger be made too prominent it may result in making them practically outcasts, to be avoided. I have seen this in other places, where people with a slight knowledge of the danger of consumptives in a community have so exaggerated matters as to forbid them in certain hotels and boarding-houses. The pamphlet should advise them as to diet, clothing, and hygiene, as well as to care with the expectoration; and I am confident this would materially add to the influences at work in the decrease of the mortality from this disease.

Also, if it were possible, for the Health Department of all large cities to undertake the thorough disinfection of all rooms in which consumptives have died, a great source of danger would be averted. In the absence of any legislation on the matter, a great deal could be done by the Health Department undertaking to do this work when requested by the householder, making a reasonable charge where possible, and in other cases doing the work free of cost. Many people would gladly avail themselves of this were it within their reach, especially as the apparatus for the work is beyond their reach. Should the Health Departments announce to the citizens that they were ready to do this work we might look for a steadily increasing demand for the use of the apparatus, and with this a lessening in the mortality.

The whole question of decrease in the death rate from Tuberculosis will depend upon proper treatment of those affected, and on the education of both the masses and the classes in the importance of prophylaxis.

I am pleased to know that Liverpool is making preparations for the reception of part of its consumptive poor in special sanatoria. This is an excellent start, and one which deserves all support. Not only will the patients there treated receive marked benefit, and many of them be able to return to their work, but what appears to me the most important function of a sanatorium will then bear fruit, that is, its work as a centre from which information regarding prophylaxis will radiate. Every patient who returns to his home will be the means of spreading amongst his own circle the knowledge of the necessity for care of a patient and his expectoration.

With the erection of these sanatoria throughout the kingdom we may hope to see in a few years most beneficial results, and Liverpool should be congratulated on taking the first step in this work.

In conclusion, I must express my thanks to Dr. Hope, the Medical Officer of Health for Liverpool, and to the officials of his department, through whom I have had access to the weekly returns of deaths covering the past five years and to other vital statistics of the city.

NOTE ON
EXPERIMENTS ON SEWAGE DISPOSAL IN GERMANY

By A. S. GRÜNBAUM, M.A., M.D., D.P.H.

During the past few years the subject of sewage disposal has received much attention in Germany. The two annual supplements to the quarterly journal of Forensic Medicine and Hygiene* contain no less than nineteen papers on this subject. The experiments have been carried out in various directions by sanitary officials and engineers, partly independently but for the greater part conjointly with Government experts, local authorities, and practising engineers. To this system of co-operative experiment by Government and municipality much of the success and reliability of the investigations is due. Nobody has any object in falsifying the results, the municipality sees that the Government has an interest in its affairs, the inventor is encouraged by the subsidy which he receives, and any improvement suggested by the official experts becomes public property, even in the case of a patented process. It seems to me an error in this country that the local authority is required to place a definite scheme before the Local Government Board, instead of the latter offering expert advice for the particular circumstances.

Most of the sewage disposal schemes which have been tried in Germany had their origin in Great Britain, but are often re-christened with a German name. Thus the closed sewage tank (so-called 'septic tank') system is known as SCHWEDER's system, and CAMERON's name is entirely omitted. The variations and changes are rather in mechanical details than of principle; into these I do not propose to enter, but to limit my descriptions to the experiments on the three newer systems of sewage disposal, omitting entirely any reference to the merely mechanical or chemical precipitation methods, since practically all of them have been found wanting.

These three systems are—

(1) Contact beds—Hamburg and Charlottenburg;

(2) Closed sewage tank (SCHWEDER's system)—Gr. Lichterfelde;

(3) Lignite paste (ROTHE-DEGENER system)—Potsdam and Tegel;

and concerning them the following notes of the results obtained may be of interest. In Hamburg, Potsdam, and Tegel I have seen the installations, but the data of all the experiments are taken from the published results.

* *Vierteljahrschrift f. gerichtl. Medicin u. oeffentliches Sanitätswesen*, Jahrgänge 1898 and 1900. Supplement.

I. CONTACT BEDS

These are only of experimental size, and, so far as I know, the system has not been tried on a large scale like in several English towns. The results are in agreement, for the most part, with those obtained in Great Britain ; but the amount of purification attained seems to have been less, and the technique of the investigation less complete. Experiments have been made with various filtering materials. The two places, Hamburg and Charlottenburg, also form a valuable contrast and comparison, since the former supplies only domestic, and the latter both domestic and trade sewage to the beds.

Hamburg

The experimental installation of contact beds (under cover) for the treating of the sewage of the General Hospital (2,000 persons) has been very thoroughly investigated by Dunbar and Zirn*, chiefly from the chemical aspect, since no bacteriological analyses are given in their published results. The sewage was purely domestic and was screened. It comes only a short distance, consequently the solids do not get much broken up. Yet with double filtration the best purification was not more than 86 p.c. diminution of oxygen absorbed, and the effluents which I saw myself were far from clear. (At Leeds, with a stronger sewage, purification up to 93 p.c. has been obtained). The authors, however, consider their results favourable to the system and draw the same conclusions as English observers, viz.: (1) clogging of the beds will occur sooner or later ; (2) the diminution in capacity, due to clogging by inorganic sludge, will not be much improved by rest ; (3) the diminution due to clogging with organic sludge will be relatively more improved by rest, but such sludge should not be allowed on to the beds in properly arranged works. The experiments with various materials all pointed to coke of appropriate size being the best. Both from a theoretical as well as from a chemical point of view, these critical and well written papers deserve careful study from those interested in the question, and it is much to be regretted that more complete chemical and some bacteriological analyses were not made.

Charlottenburg

The experimental beds had a gross capacity of 6 cbm.† and a net capacity of 5 cbm. ; they took about two hours to fill and to empty. Experiments were made with (1) coke and sand ; (2) granite, gravel, and sand ; (3) sand and brick breeze. The coarser solids were screened off.

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* *Vierteljahrschr. f. gerichtl. Medic.*, 1900 : Suppl. pp. 178 et seq.

† 1 cbm. = 1,000 litres = 220 gallons.

The conclusions which the Government experts who made the investigations arrived at may be stated as follows :—*

(1) Gravel, or gravel with brick breeze, is not an efficient medium ; coke of a definite size seems to be the best.

(2) Two hours is the best period for contact.

(3) Settling for 24 hours for 'septic' action is of no import.

(4) Cold (– 10° C.) does not interfere with the action of the beds.

(5) Aeration is necessary.

(6) The reduction in the number of bacteria is of no appreciable importance. Consequently the effluents, *if they are to enter streams, require similar precautions and treatment to merely settled sewage.*

Whilst the conclusions seem quite correct, it is evident from the protocols of the experiments that these filters were not so efficient as they can be made. Taking, for example, an experiment from the sand and coke bed after it had been working three months (Experiment III), the following numbers were obtained on analysis :—

	(Nitrogen in mgr. p. litre)				O absorbed
	Ammoniacal	Organic	Nitrate	Nitrite	Mgr. p. litre
Crude Sewage	73	13	0	0	145
Effluent (after two hours) ...	14	0	2	present	23

The absence of organic nitrogen was quite exceptional, and, although in this case the effluent was clear, it was by no means always so ; and occasionally it was putrescible.

	No. of bacteria	
	Ordinary gelatine	KI-gelatine
Crude Sewage...	2,700,000	210,000
Effluent (after two hours) ...	225,000	10,000

In many cases the plates of ordinary gelatine had liquefied (through insufficient dilution) ; the KI-gelatine is used to indicate the numbers of B. coli.

* Op. cit. pp. 262, et seq.

2. CLOSED SEWAGE TANK

GR. LICHTERFELDE (NEAR BERLIN).* *(Extracted from Official Report).*

This experimental installation was worked for over a year and then dismantled. It was merely a modification of CAMERON's system, and the effluent was run on to a contact bed. The investigation was conducted by Government officials, and the inventor was subsidised. The bacteriological and chemical analyses extended over nearly the whole of the working period. The results do not differ essentially from those obtained elsewhere. The alteration in quantity of solids in suspension in the effluent is not recorded ; the total solids increased rather than diminished.

On dismantling the closed tank it was found that sludge had accumulated to the extent of 13.32 cbm., the total capacity of the tank being 90 cbm. The sludge did not differ chemically from that obtained from fresh sewage. Consequently *no '**mineralisation**' had occurred.* The dilute nature of the sewage accounted for the sludge not having made itself very evident during the working period. In another installation the sludge had to be removed twice within six months from the tank.

The experiment showed the possibility of dealing with the sludge of a dilute sewage for a certain time without nuisance ; and the system may be useful for hospitals, barracks, &c.

These results are hardly so good as those obtained in England. But they all point in the same direction, viz., that, except to avoid smell, the closed presents no advantage over the open sewage tank, whilst it is considerably more expensive, and it does not in many cases dispose of the sludge in the effectual way that was expected and affirmed.

3. THE LIGNITE PROCESS (ROTHE-DEGENER PATENT)

POTSDAM AND TEGEL

I do not know of any similar process in use in this country. The sewage is first mixed with crushed lignite and then precipitated with ferric sulphate. The sludge settles and the effluent is drawn up into a Rothe-Roeckner tower. The former is drawn off from below, pressed, and finally used as fuel in the boiler furnace ; the latter is mixed with a small quantity of bleaching powder, and, thus sterilised, is run into the river. The free chlorine can, if desired, be easily removed by filtering through a shallow bed of coke or gravel.

* Op. cit. pp. 288 et seq. ; also 1898, Suppl., pp. 99 et seq.

The quantities used are : 1 kg. lignite to 1 cbm. sewage and then 170 grammes ferric sulphate, and finally bleaching powder to the amount of 0.15 ‰.

As examples of the results the following, taken at random, may be quoted :—

	Ammon. N.	Organic N.	Oxygen absorbed
Crude Sewage	92.4	33.6	409.55
Settled „	50.4	8.4	101.86

	Ordinary gelatine	KI-gelatine
Crude Sewage...	Liquefied	320,000
Settled „	„	140,000
Bleaching Powder „	Sterile	Sterile

The effluent was not putrescible. Allowing the effluent to go through a filter bed usually caused a re-appearance of **B.** coli ; and since running the chlorine-containing effluent straight into the river (Havel) caused no harm to the flora and fauna, nor even appreciably affected the amount of chlorides, the filtration seems to be superfluous.

This process is of considerable importance because it demonstrates the practicability of obtaining a bacteriologically perfect effluent, and of disposing of the sludge. The cost is rather considerable (1s. 3d. per head), but it seemed to me that the mechanical arrangements might be simplified and made more economical. In Potsdam domestic sewage from over 50,000 inhabitants is treated in this way.*

The problem of 'sewage disposal' does not present so many difficulties in Germany as in this country. The rivers are mostly of greater size, and there is more land available for sewage farms. According to Mr. LINDLEY, who has probably the greatest experience in the construction of sewage schemes, it would require three of the largest English rivers in their non-tidal portion to make a volume equal to that of the Main at Frankfurt. It is, consequently, more easy to obtain the minimum dilution of 1 in 16 necessary to obviate putrefaction. In many instances the dilution reaches 1 in 1,000, or more, so that putrefaction hardly enters into consideration, and the deposit of solid matter on the banks only occasionally. Nevertheless, the undesirability of allowing untreated sewage to enter even a large river is being recognised, and the city of Cologne has been compelled to erect works for treating its sewage.

* Whittaker and Ducat Filters have not, so far as I am aware, been tried anywhere in Germany.

Yet the legal enactments regarding sewage disposal are hardly so definite as in this country. There is no special Act relating to Rivers Pollution, and local by-laws cannot exceed the common law. Moreover, there is an unfortunate decision of the Supreme Court of June 2nd, 1886, which says that a pollution not exceeding what is customary ('gemeinueblich') must be permitted, even if it damage the utility of the water. Consequently there is no standard, and each case is judged upon its merits. There are, however, indications of impending changes and improvement.* A committee of experts has been formed, with official recognition, for the complete (biological, chemical, &c.) investigation of rivers, and there is a possibility of legislative regulations being introduced in the near future.

It has been possible to lay down certain rules without statutory authority. Thus, in all cases where the sewage is treated by chemical means, the effluent is required to be free from B. coli. But this condition has not been applied to other methods of treatment, and no regulations have been laid down for sewage farms. Consequently the sewage fungus Leptomitus occurs in the effluents from the Berlin sewage farm, and it grew so luxuriantly that a bathing place in the River Panke, which received the effluent, became unusable. Yet the owner could obtain no redress, although he tried to do so, in face of the decision of 1886.

On the other hand certain trades, and more particularly the sugar refineries, have been compelled to purify their waste; and this has been done without any obvious detriment to the trade. A friendly co-operation between manufacturers and the Government exists, and advice and direction are given in each individual case by a committee composed of a commissary and an expert of the State and of the Directorate of the Union of German Sugar Industry. In spite of all these experiments it is, of course, recognised that, where possible, the proper method of sewage disposal is on a farm, and that only when this is unattainable, should other methods be adopted.

Although it would appear that, both in technique and in results, the investigations undertaken in Germany fall behind those of this country, it is the harmonious association, to which allusion has been several times made, between the Administration and the municipality or the trade, the readiness of the Government to give advice instead of only criticism, which forms the most pleasant and striking feature of the present experimental stage of sewage disposal in Germany. To quote the words† of Dr. SCHMIDTMANN, the principal medical officer of the Cultus Ministerium :—

'This appropriate union of State and private objects and interests, the definite co-operation of science, technique and practice, promises most successful results,

* Op. cit. 1900, Suppl., p. 308.

† Op. cit. 1900, Suppl., p. 319.

and puts before us a picture of how in the future the great economic questions regarding water supply and sewage disposal, as yet unanswered, may be solved. The direct participation, by the experts of the State departments concerned, in the foregoing problems, has to a large extent put an end to existing uncertainty and given stimulus and support to purposeful honest exertion and work, and, with comparatively small sacrifice on the part of the State, the latter has been able to make use of the large means at the disposal of the municipalities and trades concerned for the common weal.'

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